A Review of Depleted Uranium Biological Effects: *In vivo* Studies

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Report Documentation Page

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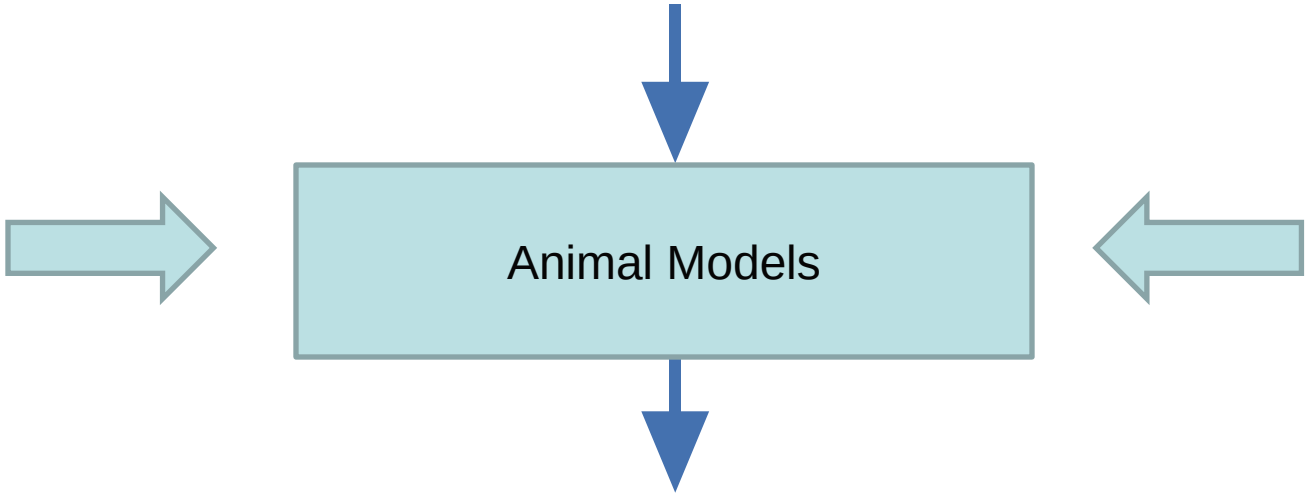
Research Approach:
Follow Regulatory Agency Approach

IARC,NTP, FDA, EPA

Carcinogenic Hazard Evaluation

Transformation + Mutagenicity + Cytogenicity



Human Epidemiology

“All models are wrong but some are useful”

Dr. George Box, Statistician, Mathematician

Problems in Evaluating Carcinogenicity

1) **Epidemiology studies alone are not adequate – too few participants, confounders**

2) **Animal studies alone are not adequate, relevance to humans, dose/dosing**

3) **Cell studies alone are not adequate; relevance to humans,**

Examples: asbestos (animal studies ignored)
saccharin (rodent carcinogen, not human)
diethylstilbestrol (rodent model selection; ignored cell studies)

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

1) **Is long-term exposure to internalized DU toxic or carcinogenic?**

2) **Does DU cause transgenerational effects?**

3) **Does DU cause radiation effects?**

4) **Can we distinguish between DU and other exposures (radiation, chemical)?**

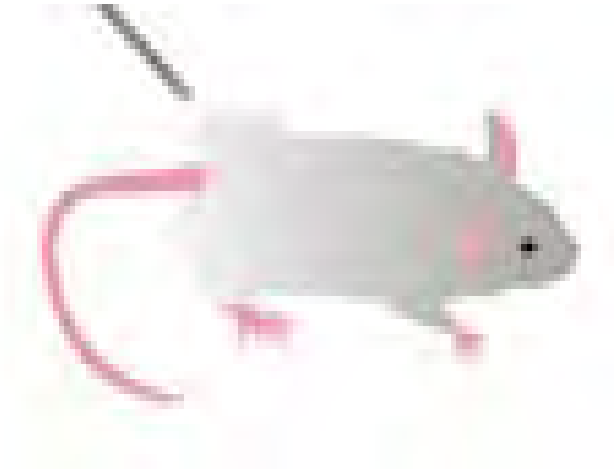
Toxicology *in vivo*
DU Chronic Internal Exposure
via Implanted DU

- Sprague-Dawley Rat
- Implanted DU Pellets in Hind Limbs
- DU Pellet: 1mm diameter, 2 mm long
- Serial Euthanasia 6, 12, 18 months

In Vivo Model

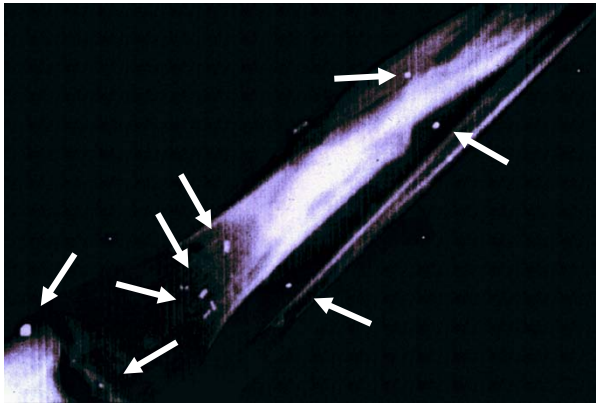
Implantation of DU Pellet

Relationship of Rodent DU Implants To Human DU Exposure (Wounding)



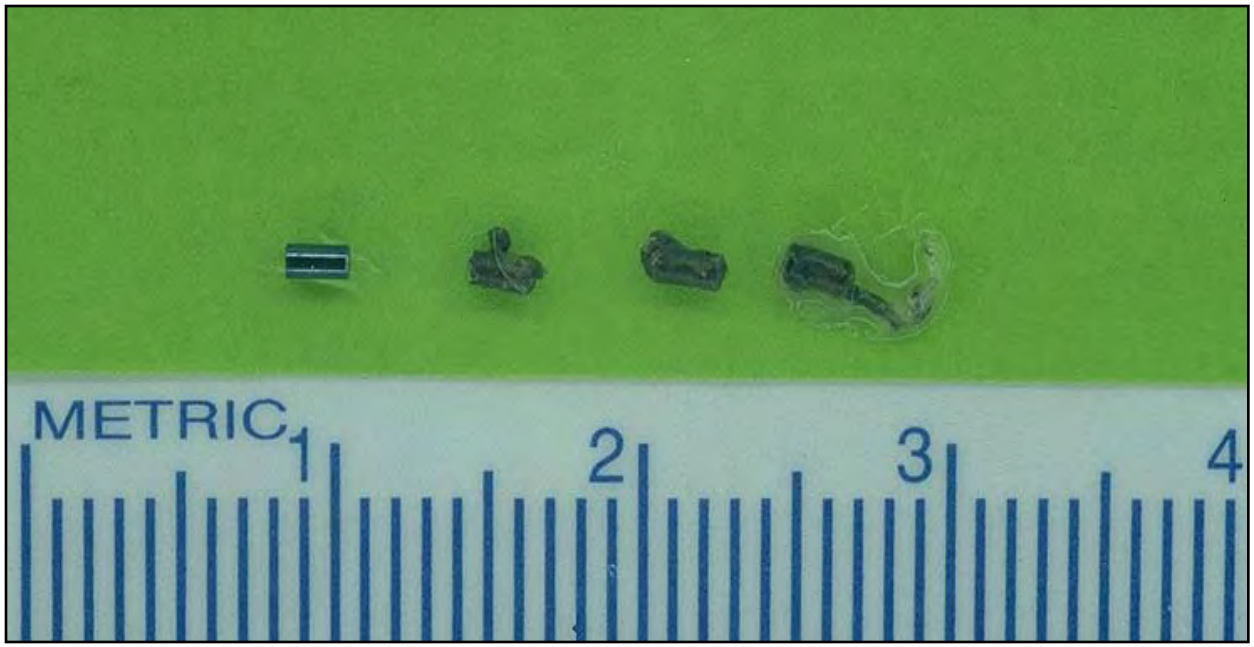
High Dose
20 or 6 pellets

↕ ↕



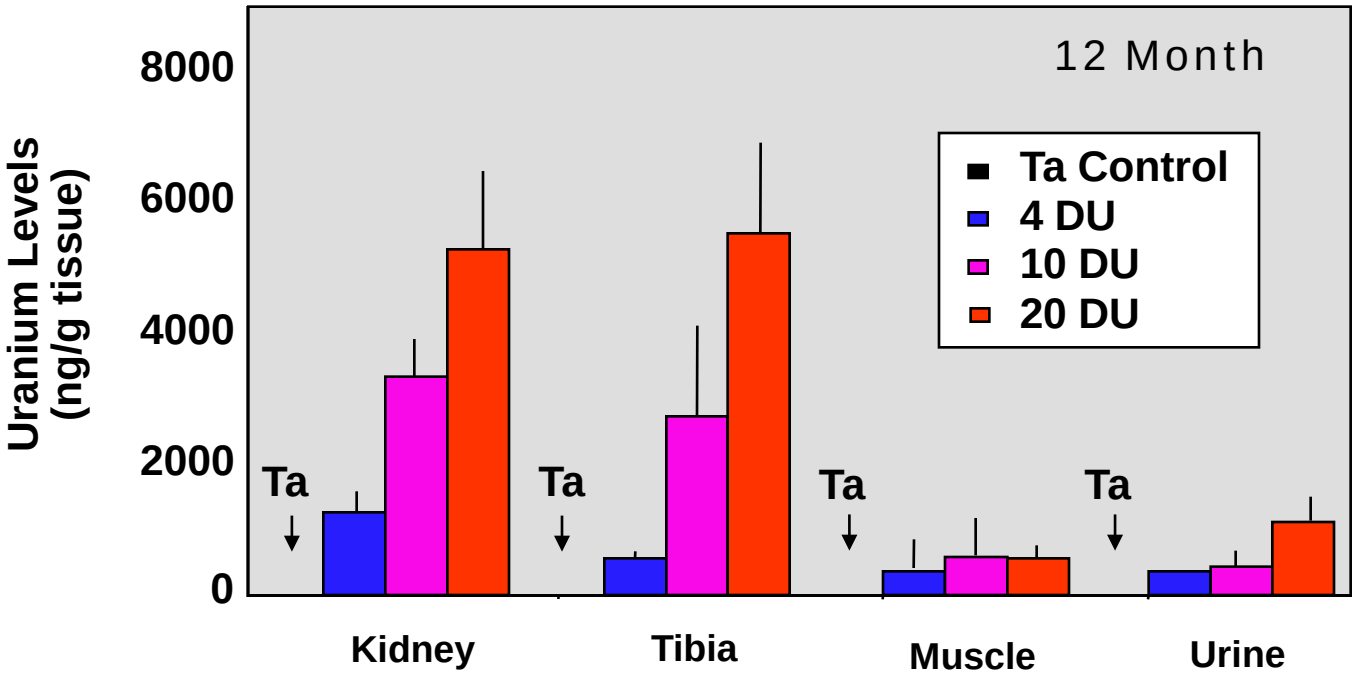
Highest DU Excretor

Health Effects of Embedded Depleted Uranium



DU Pellet Implants
New and at 90 days

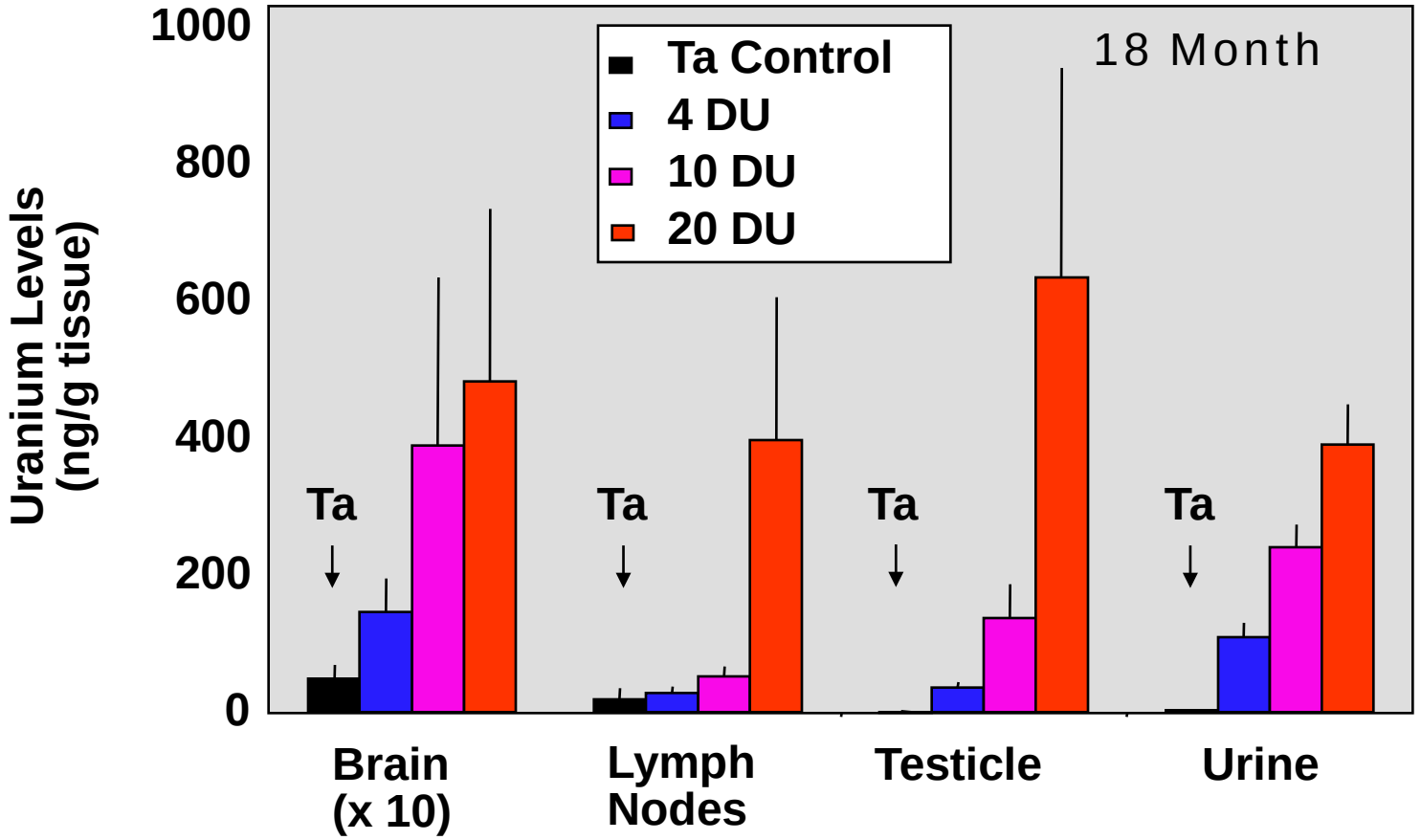
Uranium Distribution in Rat Tissue after DU Pellet Implantation



- ***Uranium redistributes with time to various organs and tissues, especially bone and kidney***
- ***No apparent changes in bone histology***

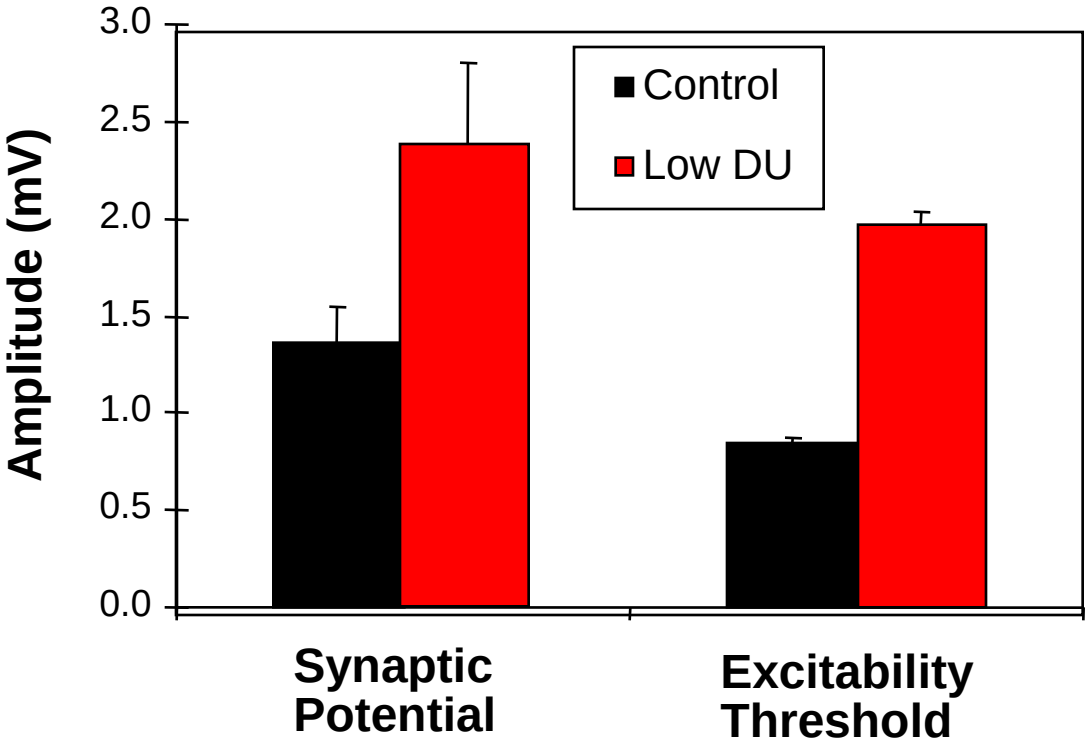
Pellmar *et al*., *Toxicol. Sci*. 49, 29-39 (1999)

Uranium Distribution in Rat Tissue after DU Pellet Implantation



Neurotoxicity

Neurotoxicity 1 Year After DU Pellet Implantation

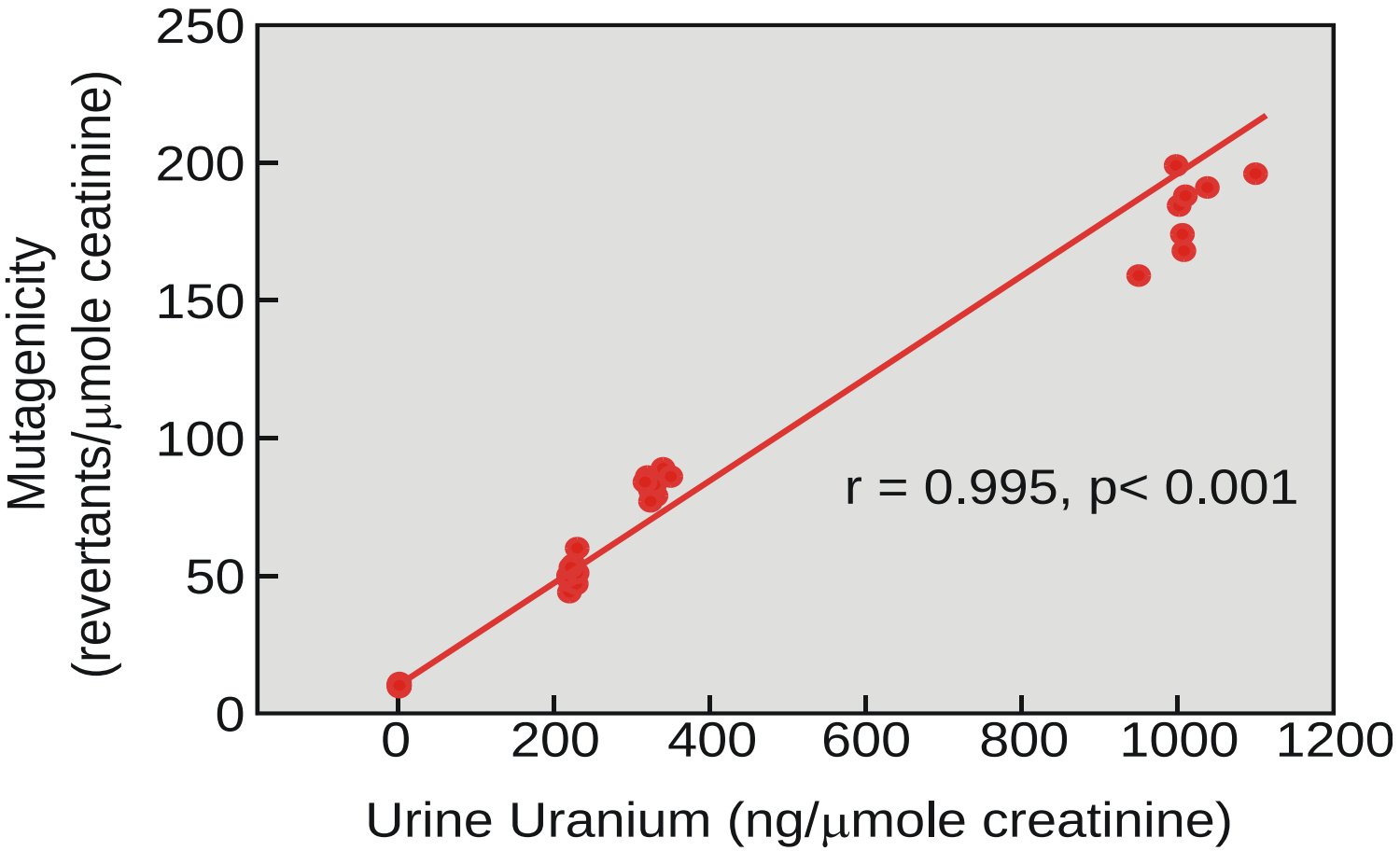


Pellmar et al, *Neurotoxicology* 20(5), 1999

Mutagenicity

Correlation Between Urinary Mutagenicity and Urinary Uranium Content

(12 Months Post DU Implantation)



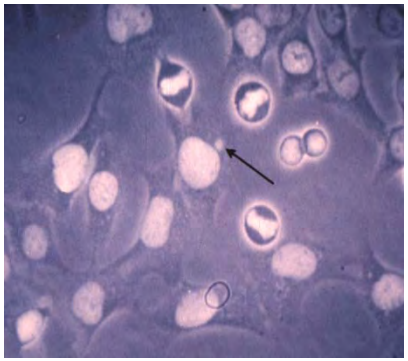
Miller *et al*., *Mutagenesis*, vol., 13, no. 6, 643-648 (1998)

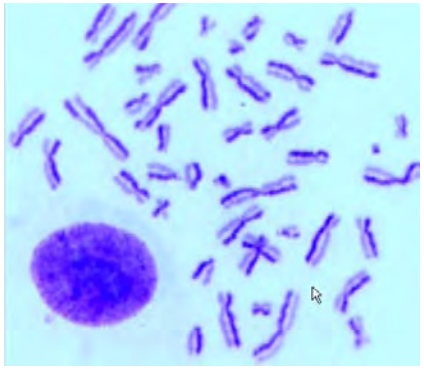
DU Genotoxicity *in vivo*

(Sprague-Dawley Rats)

Genotoxicity in DU-Implanted Rats
18 months post-implantation

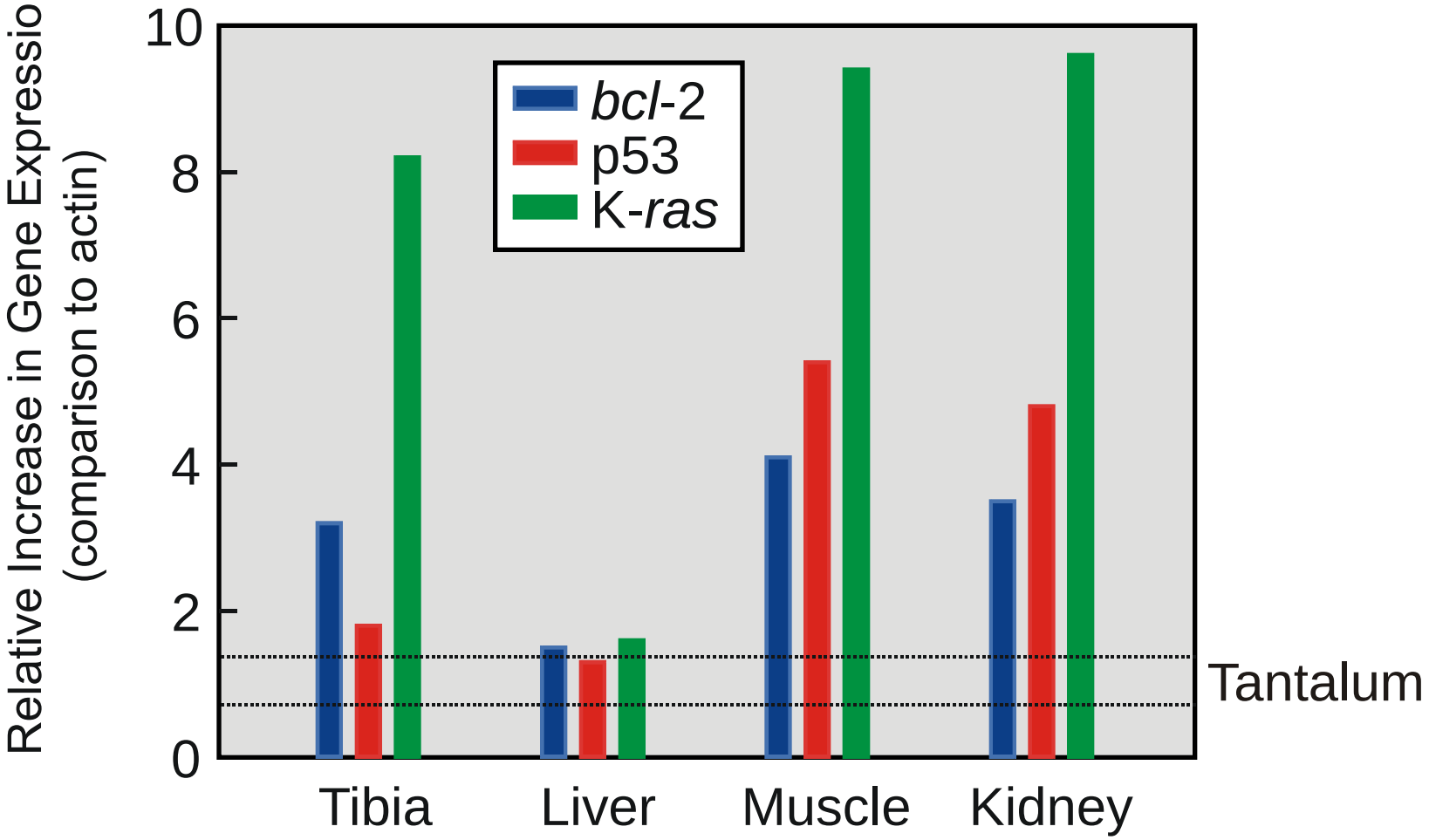
Genotoxic Measure	Treatment	Frequency (%)	
Micronuclei	Control	0.31	0.03
	DU	0.55	0.05
	Ta	0.29	0.03
SCE	Control	0.40	0.04
	DU	0.91	0.08
	Ta	0.36	0.04
Chromosomal Aberrations	Control	0.25	0.03
	DU	0.49	0.05
	Ta	0.29	0.03





Miller et al, 2003, *Mil Med.* 2002 Feb;167(2 Suppl):120-2.

Genomic Effects of DU
Oncogene and Tumor Suppressor Alterations



Miller *et al*., *Cancer Detection and Prevention*, Vol 20, Issue 5, 528-529, 1996.

Conclusions from Toxicology Study of Embedded DU in Rat

- **DU distributes to organs**
- **Uranium retention is long-term**
- **DU causes some neurotoxicity**
- **DU induces genotoxicity**
- **DU urine is mutagenic**
- **DU causes adverse oncogene changes**

Toxicology *in vivo*
DU Inhalation Studies

French Institute Nuclear Security
Pierrelatte, Fr
Fountenay au Roses, Fr

Genotoxic and Inflammatory Effects of Depleted Uranium Particles Inhaled by Rats

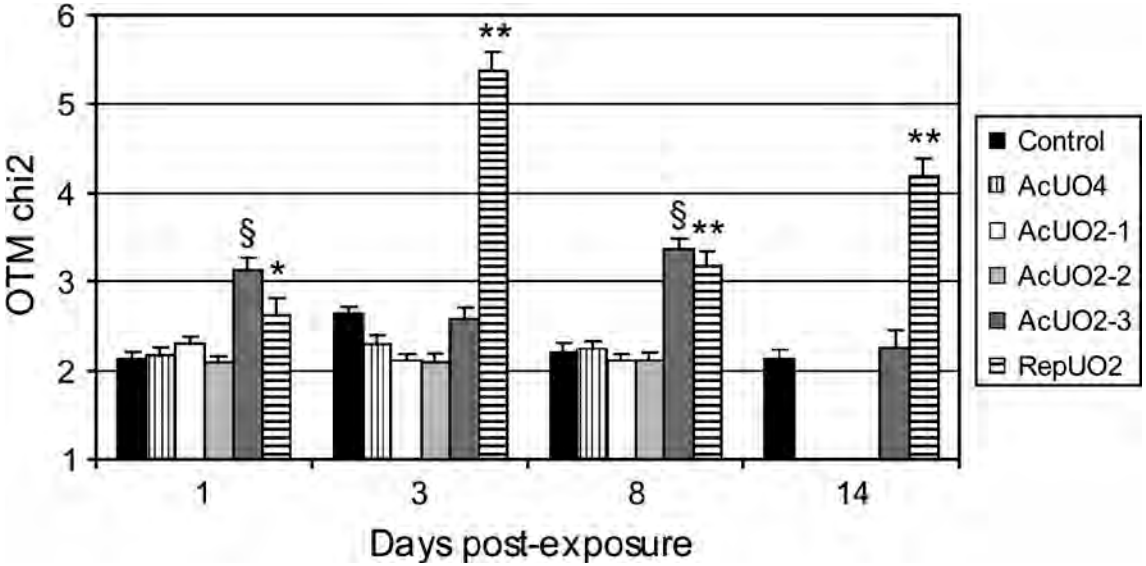
Experimental Protocol for Inhalation Study

•Note. h, hours; min, minutes.

Inhalation type	Group name	Inhalation duration	Aerosols concentration	Euthanasia post-exposure
Acute UO_2	AcUO2-1	30 min	190 $mg.m^{-3}$ ± 41 $mg.m^{-3}$	4 h, 1, 3, and 8 days
Acute UO_2	AcUO2-2	2 h	375 $mg.m^{-3}$ ± 70 $mg.m^{-3}$	1, 3, and 8 days
Acute UO_2	AcUO2-3	3 h	375 $mg.m^{-3}$ ± 70 $mg.m^{-3}$	1, 3, 8, and 14 days
Repeated UO_2	RepUO2	30 min, 4 days/week, for 3 weeks	190 $mg.m^{-3}$ ± 41 $mg.m^{-3}$	1, 3, 8, and 14 days
Acute UO_4	AcUO4	30 min	116 mg/m^3 ± 60 $mg.m^{-3}$	4 h, 1, 3, and 8 days
Air	Control	30 min, 4 days/week, for 3 weeks	Air	1, 3, 8, and 14 days

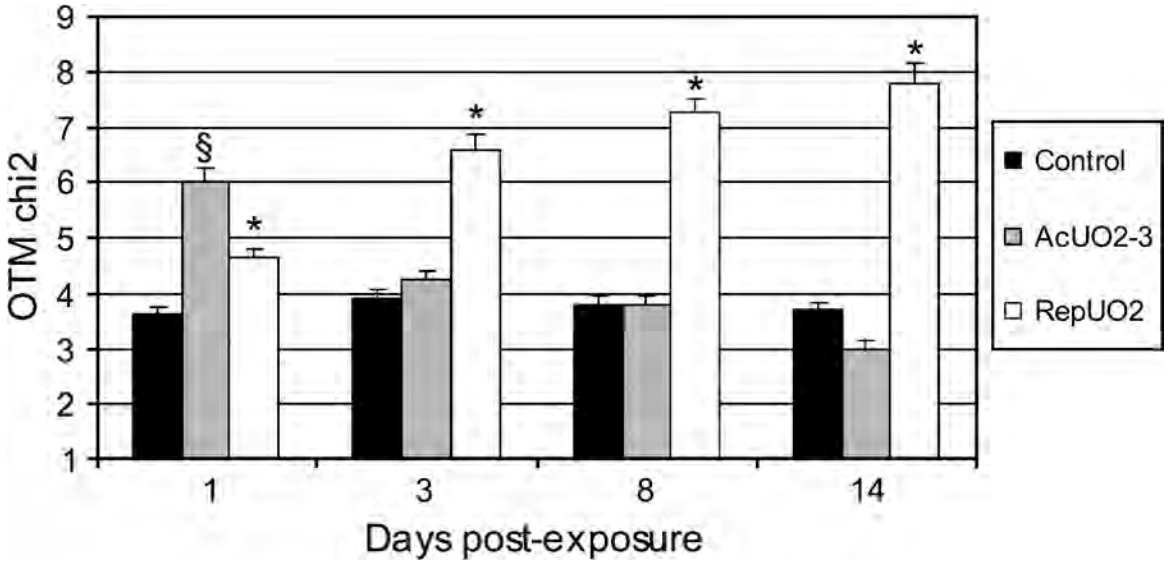
Monleau et al, 2006; ***Toxicol Science.*** Jan;89(1):287-95.

Genotoxic effects of depleted uranium particles inhaled by rats.



Comet assay in Bal cells
DNA strand breaks

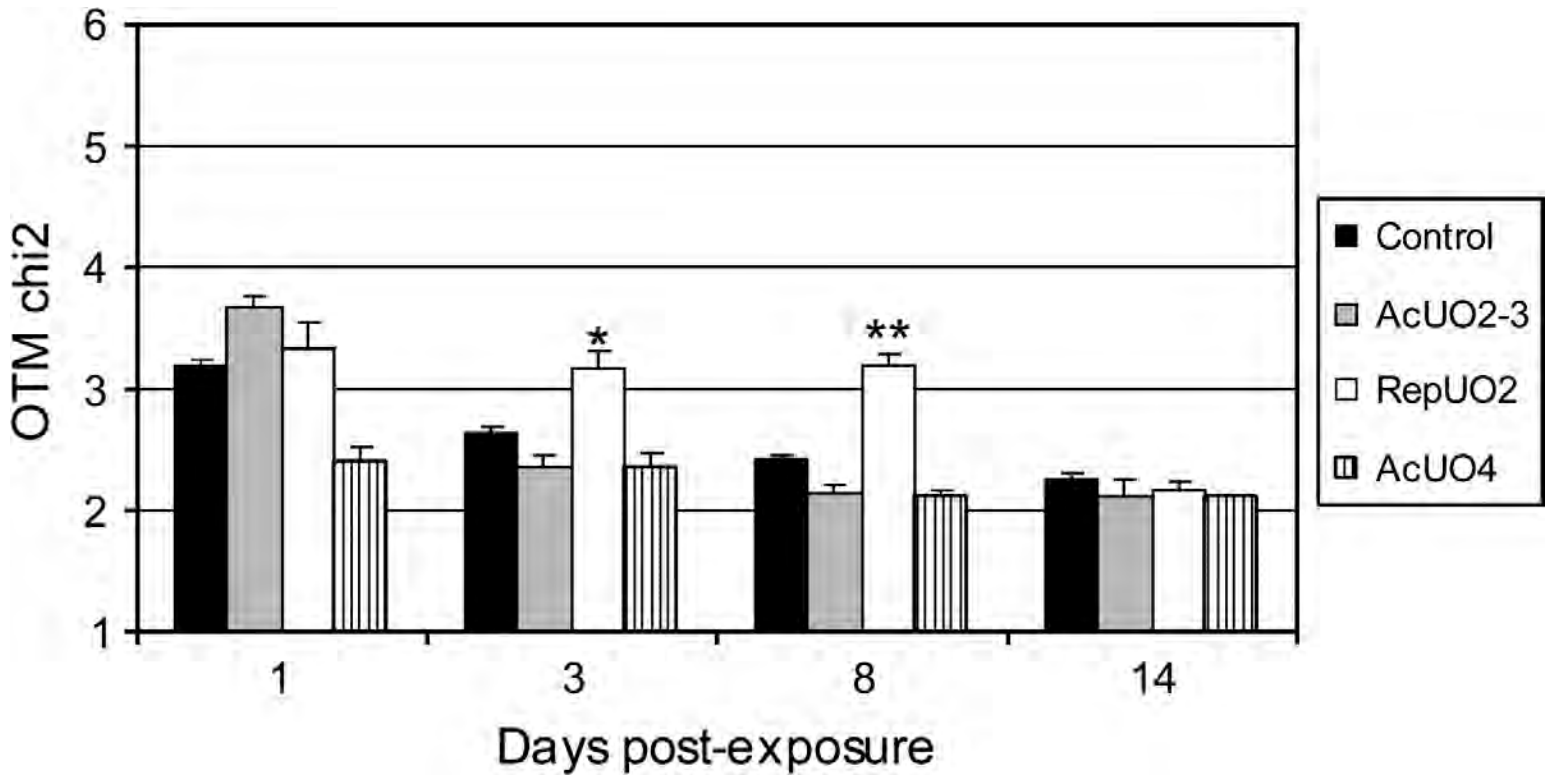
Comet assay in Bal cells
DNA double strand breaks



Monleau M et al. Toxicol. Sci. 2006;89:287-295

Genotoxic effects of depleted uranium particles inhaled by rats.

Comet assay in Kidney cells - DNA strand breaks



Findings: Repeated Inhalations Most Genotoxic

Monleau M et al. Toxicol. Sci. 2006;89:287-295

DU Toxicology Studies: Oral Administration

Renal Anemia Induced by Chronic Ingestion of Depleted Uranium in Rats

DU Effects on Hematological Parameters

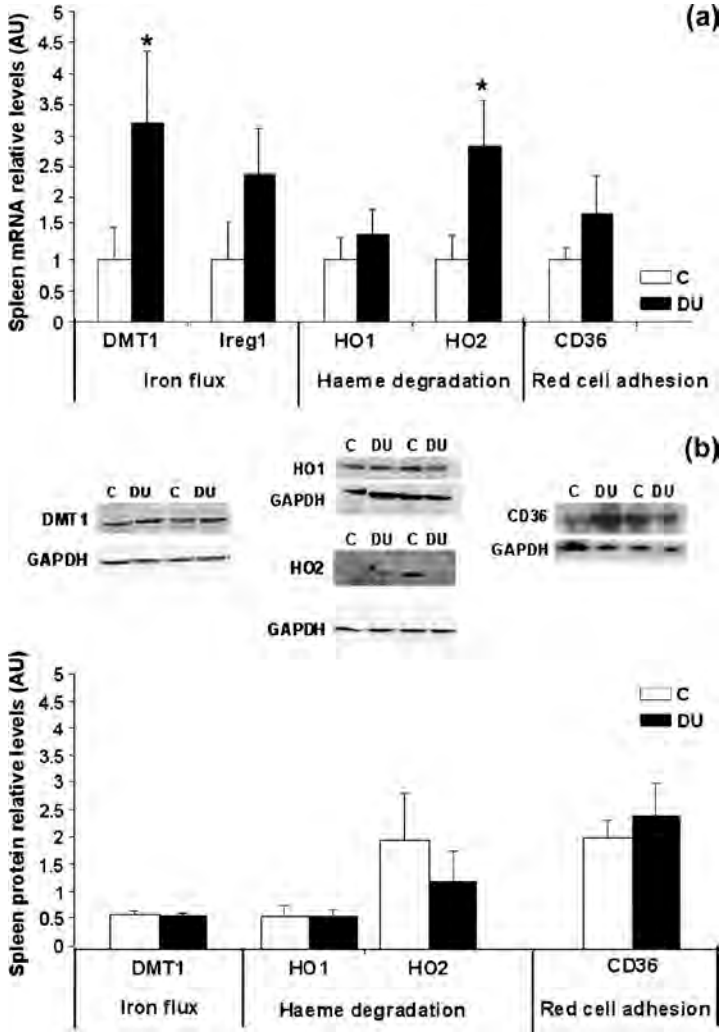
	Control (n = 7)	DU (n = 8)	p
Complete blood count			
RBC (10^{12}/l)	10.2 ± 0.5	8.3 ± 0.7	0.048*
Hemoglobin (g/l)	15.8 ± 1.8	12.8 ± 2.5	0.056
Hematocrit (%)	55.9 ± 3.1	45.5 ± 3.7	0.051
Leucocytes (10^{9}/l)	5.1 ± 0.9	5.2 ± 1.0	ns
Iron and binding capacity			
Iron concentration (µmol/l)	48.9 ± 7.4	39.5 ± 5.6	ns
UIBC (µmol/l)	90.2 ± 19.1	81.8 ± 8.6	ns
TIBC (µmol/l)	139 ± 15	121 ± 13	ns

Berradi H et al. *Toxicol. Sci*. 2008;103:397-408

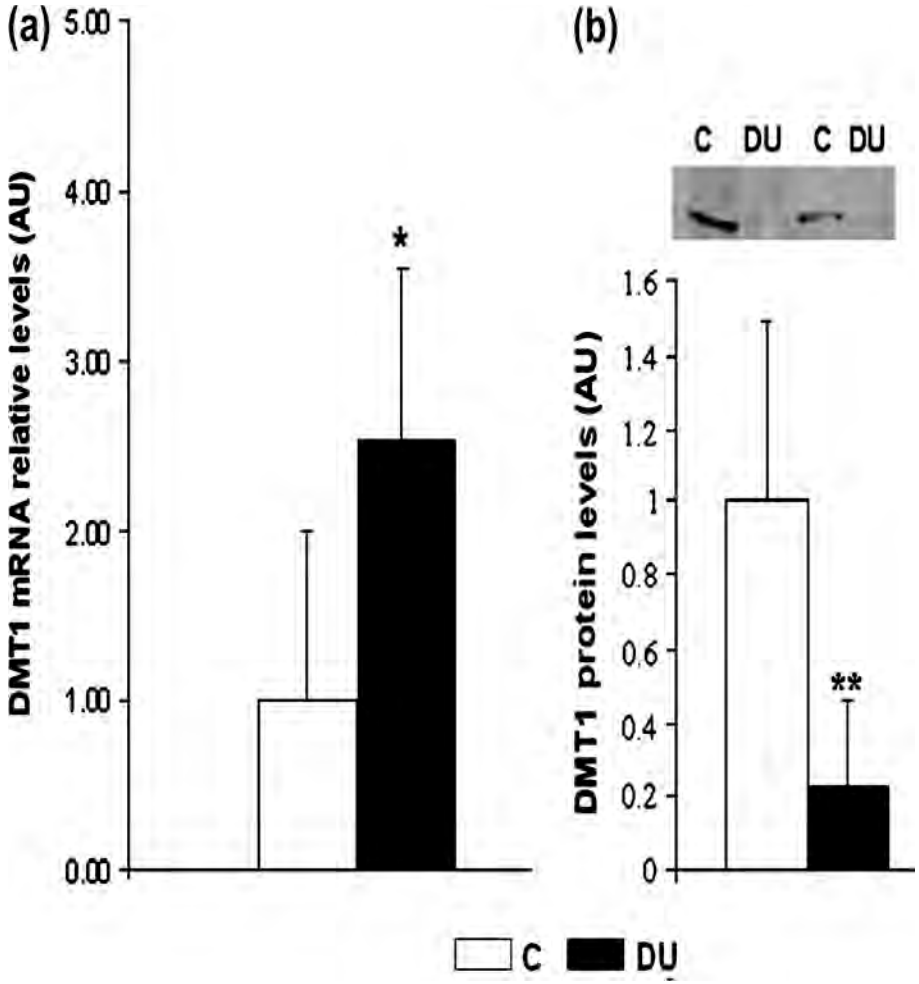
- ***Note. Statistical analyses were performed using Student t-test. *p < 0.05 significantly different from control group.***
- ***A significant decrease was observed in RBC number.***
- ***Hemoglobin and hematocrit also tended to diminish after chronic DU ingestion. ns, not significant.***

Oral Administration of DU Induces Renal Anemia

Changes in expression of splenic iron recycling markers.



Renal expression of iron transporter DMT1 in DU-contaminated and control rats.



Berradi H et al. Toxicol. Sci. 2008;103:397-408

Summary
Evaluation of Oral or Inhaled DU Exposure on Rat Behavior, Toxicological Endpoints, Offspring Effects

Uranium Distribution	Behavior; Locomotor	Brain Accumulation	Sperm Effects	Immune Effects	Offspring Effects Behavior
Yes	Yes	Yes	No effects	Decreased Inflammatory pathways	F1 no cancers
Time and Dose Dependent	Time and Dose Dependent	Exposure type dependent		Decreased macrophages, mast cells	F1 ↑ hyperactivity

French Institute Nuclear Security (IRSN)

Muller M, et al., 2008 , Uptake of Uranium, via endocytosis. *Toxicol Sci*. Feb;101(2):254-62

Dublineau I, et al, 2007, Modifications of Inflammatory pathways in intestine by DU . *Toxicol Sci*. 2007 Aug;98(2):458-68

Monleau, M, et al. 2006. Bioaccumulation and behavioural effects of depleted uranium in rats exposed to repeated inhalations.

Houpert P et al, 2005 Heterogeneous accumulation of uranium in the brain of rats**.** *Radiat Prot Dosimetry*. 2007;127(1-4):86-9.

Lestaevel P, et al., 2005. Brain is a target organ for DU*. Toxicology.* Sep 1;212(2-3):219-26.

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

1) **Is long-term exposure to internalized DU toxic or carcinogenic?**

2) **Does DU cause transgenerational effects?**

3) **Does DU cause radiation effects?**

4) **Can we distinguish between DU and other exposures (radiation, chemical)?**

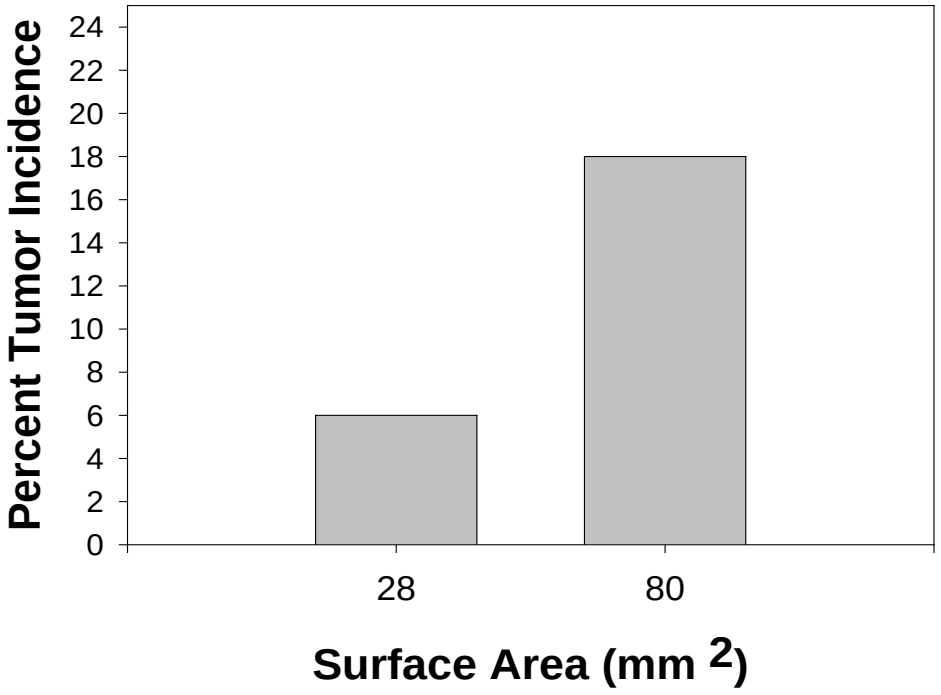
AFRRI DU Carcinogenicity Study

Dr. Kalinich

DU Carcinogenicity

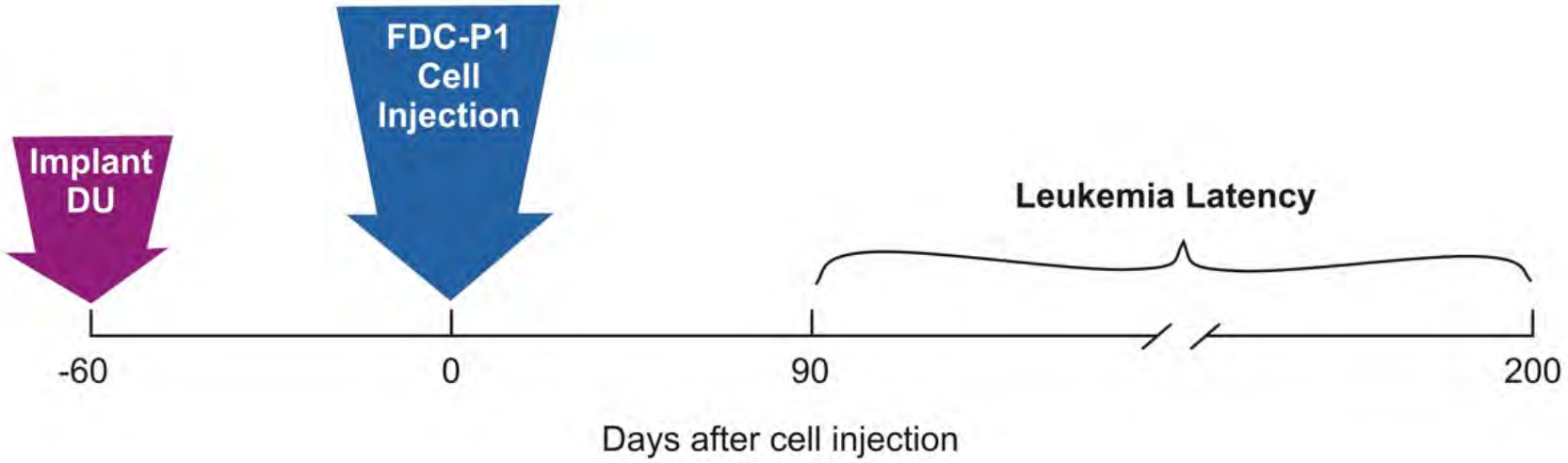
1995 MRMC-funded Study

Incidence of Soft Tumors in Rats Implanted with DU

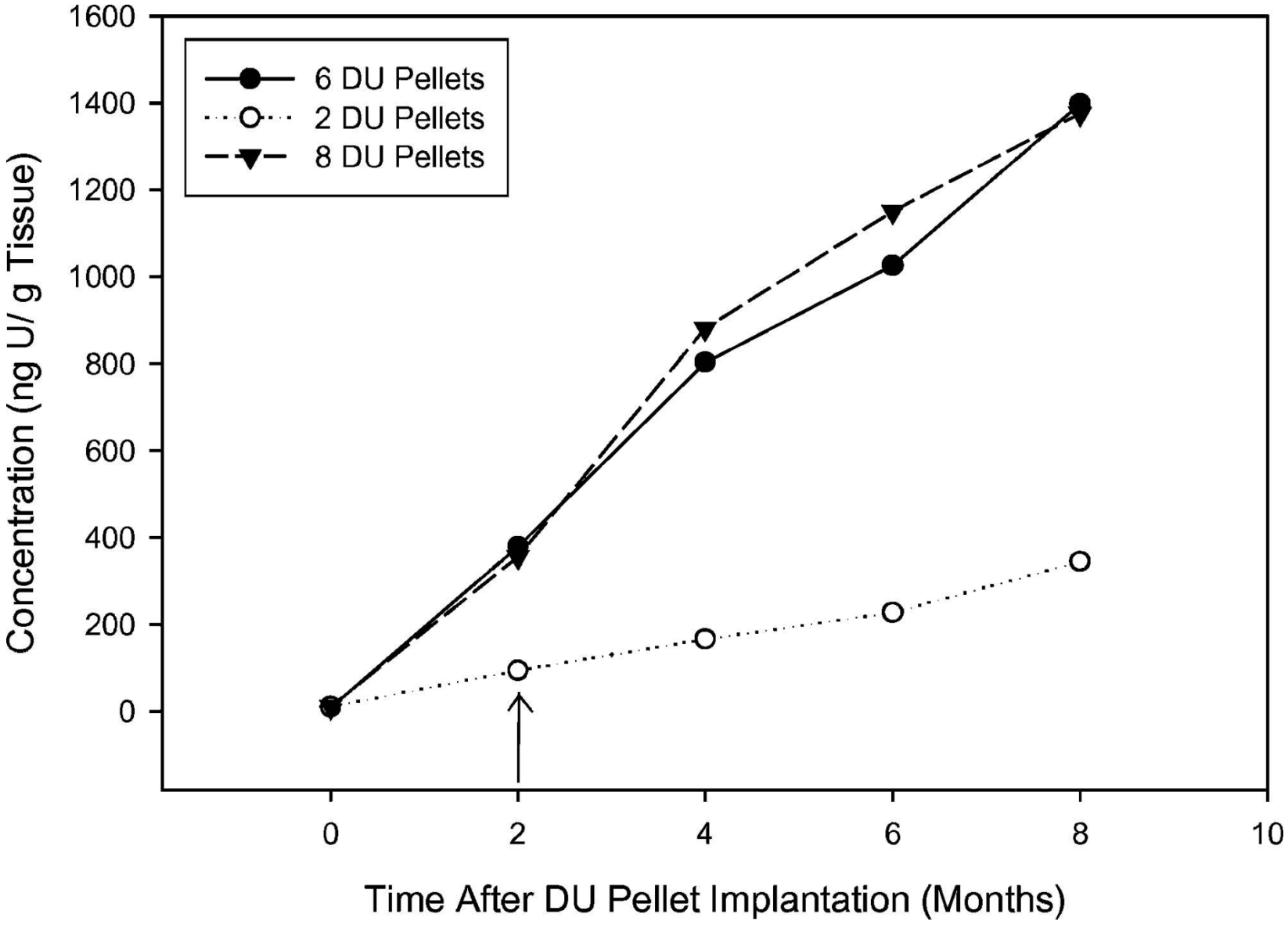


Hahn, et. al.,. *Environmental Health Perspectives,* 2002 Jan;110(1):51-9.

Model to Study Depleted Uranium-Induced Leukemia



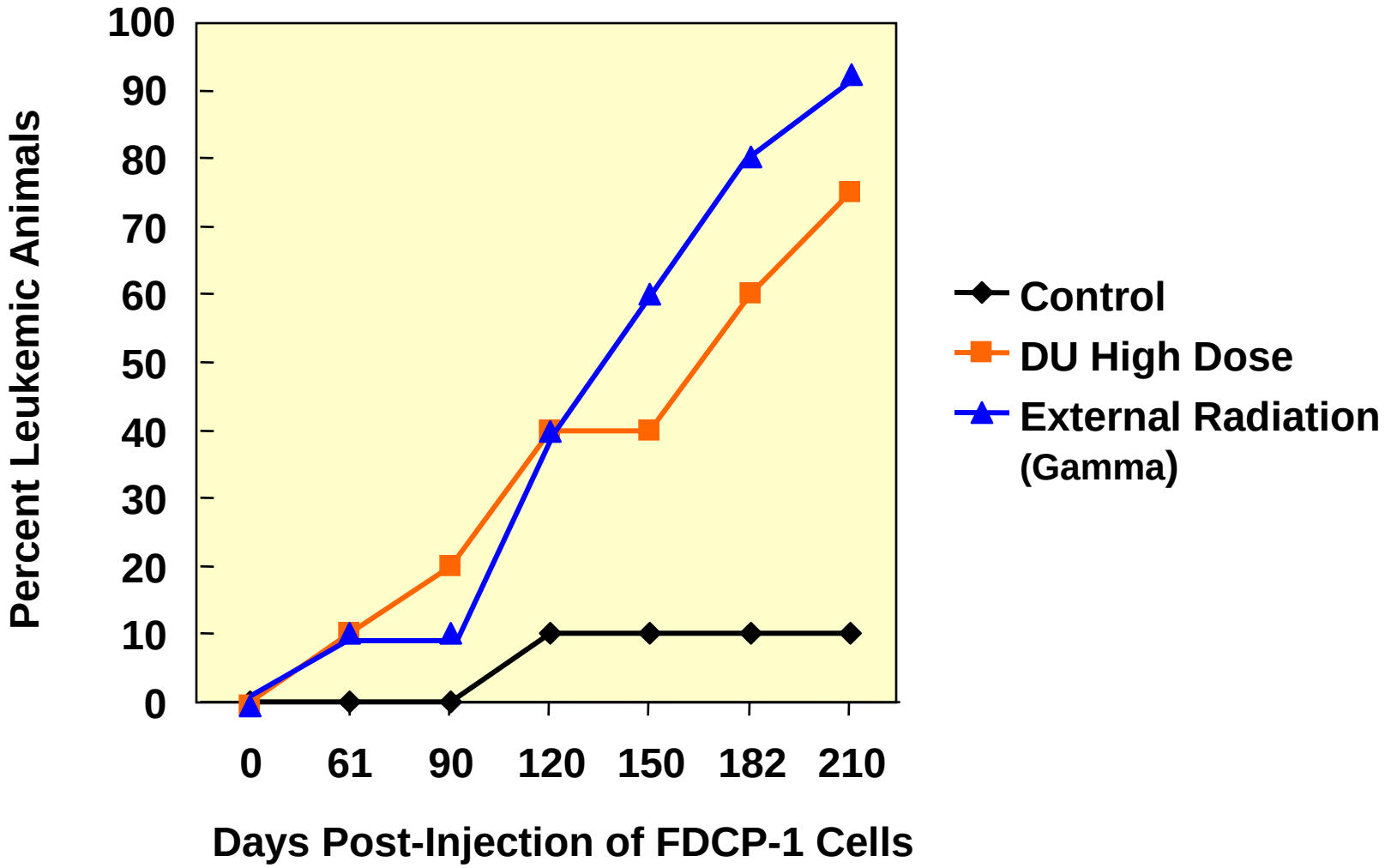
Bone Marrow Uranium Levels in DU-Exposed Mice



Miller et al, *Molecular and Cellular Biochemistry*, Nov;279(1-2):97-104 (2005).

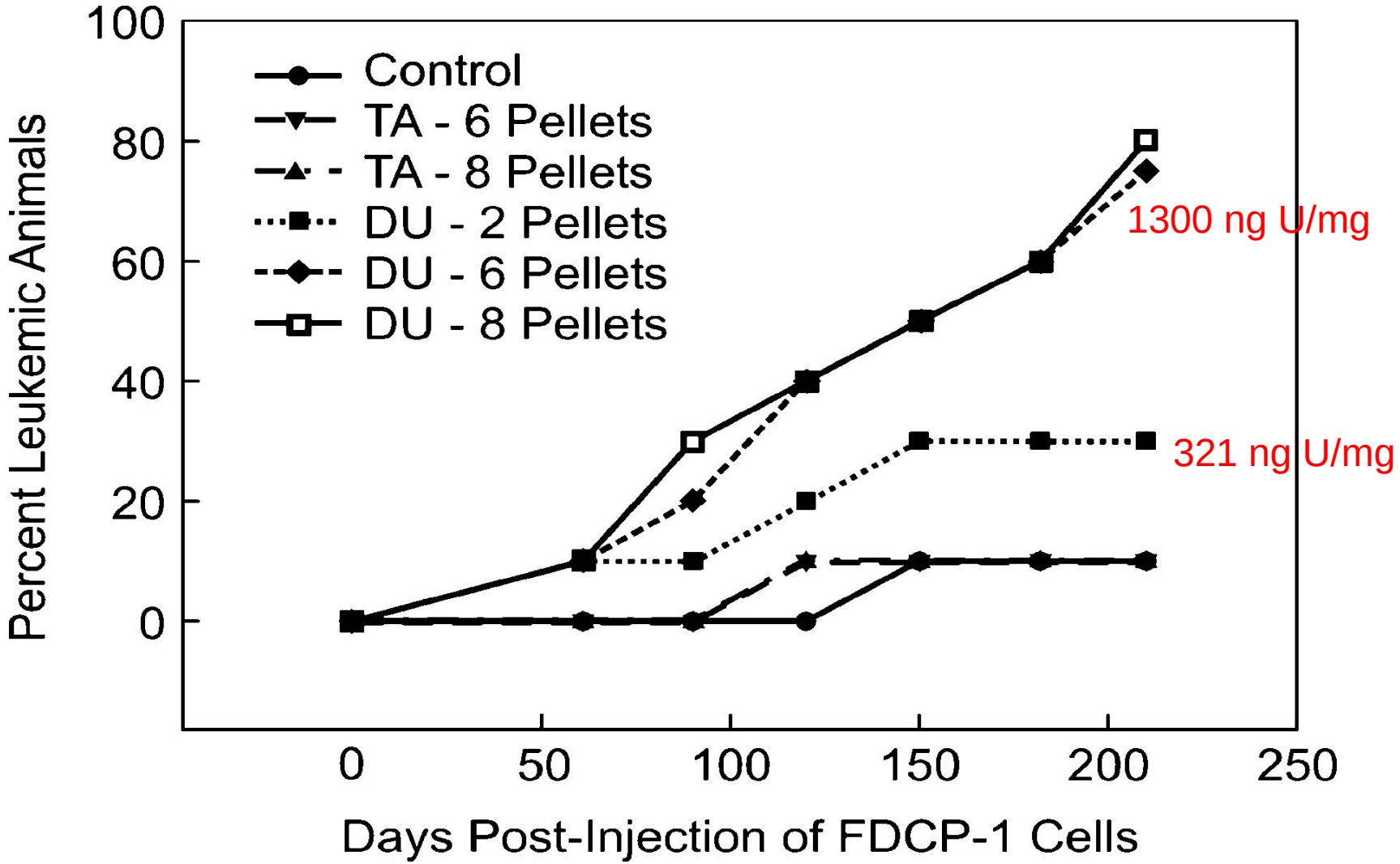
DU Carcinogenicity/Leukemogenesis *in vivo*:

DU-Induced Leukemia after Exposure to Embedded DU for 60 Days



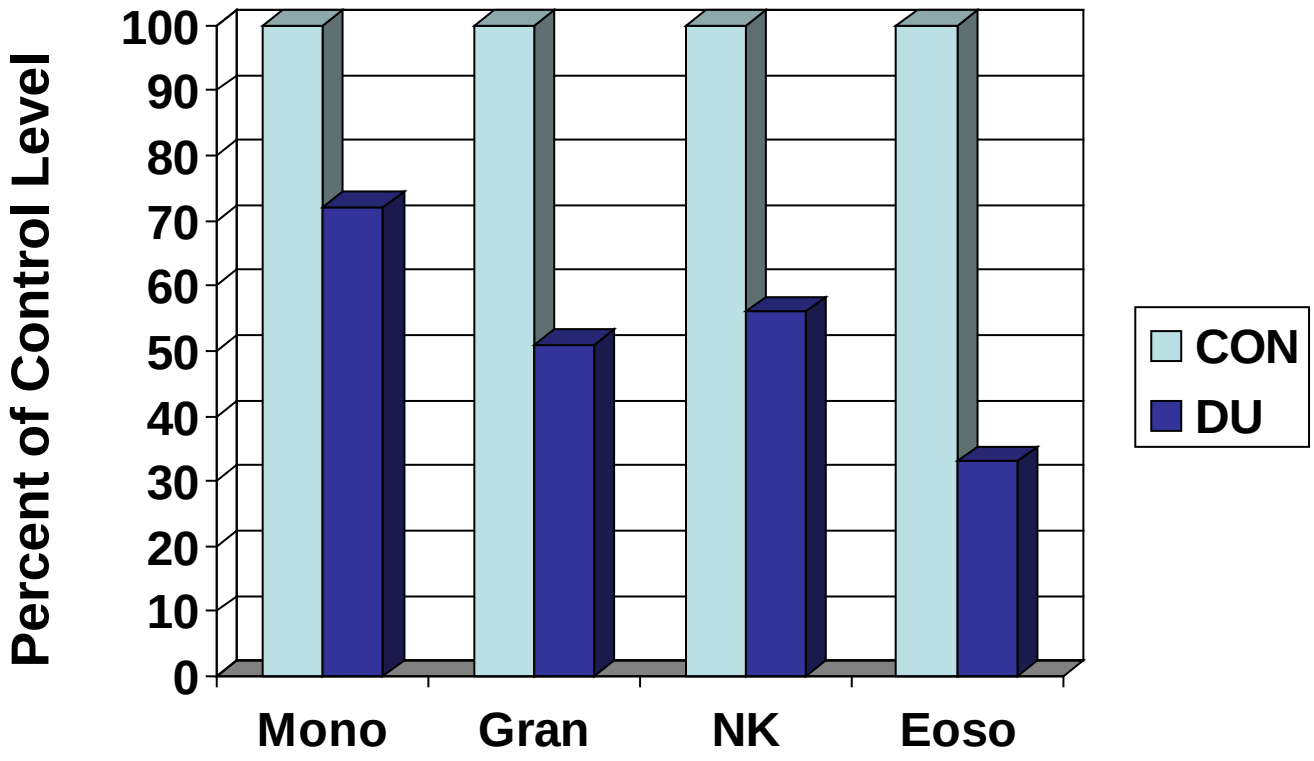
Miller et al, ***Molecular and Cellular Biochemistry***, Nov;279(1-2):97-104 (2005).

Incidence of DU-Dose on Leukemia Incidence Uranium Concentration-Dependent

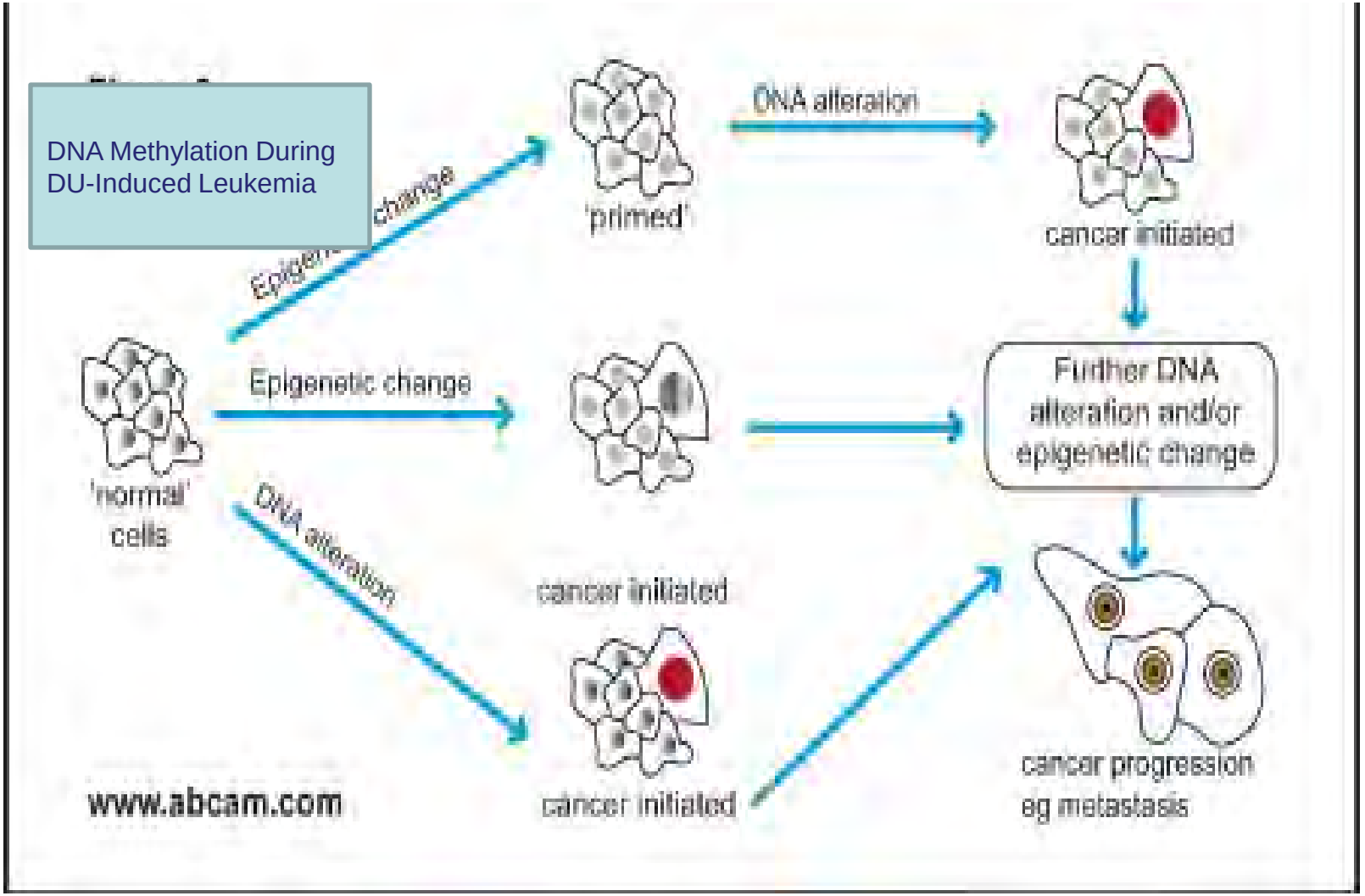


Miller et al, *Molecular and Cellular Biochemistry*, Nov;279(1-2):97-104 (2005).

Effect of DU on Blood Element Counts:
60 Days Post-Pellet Implantation



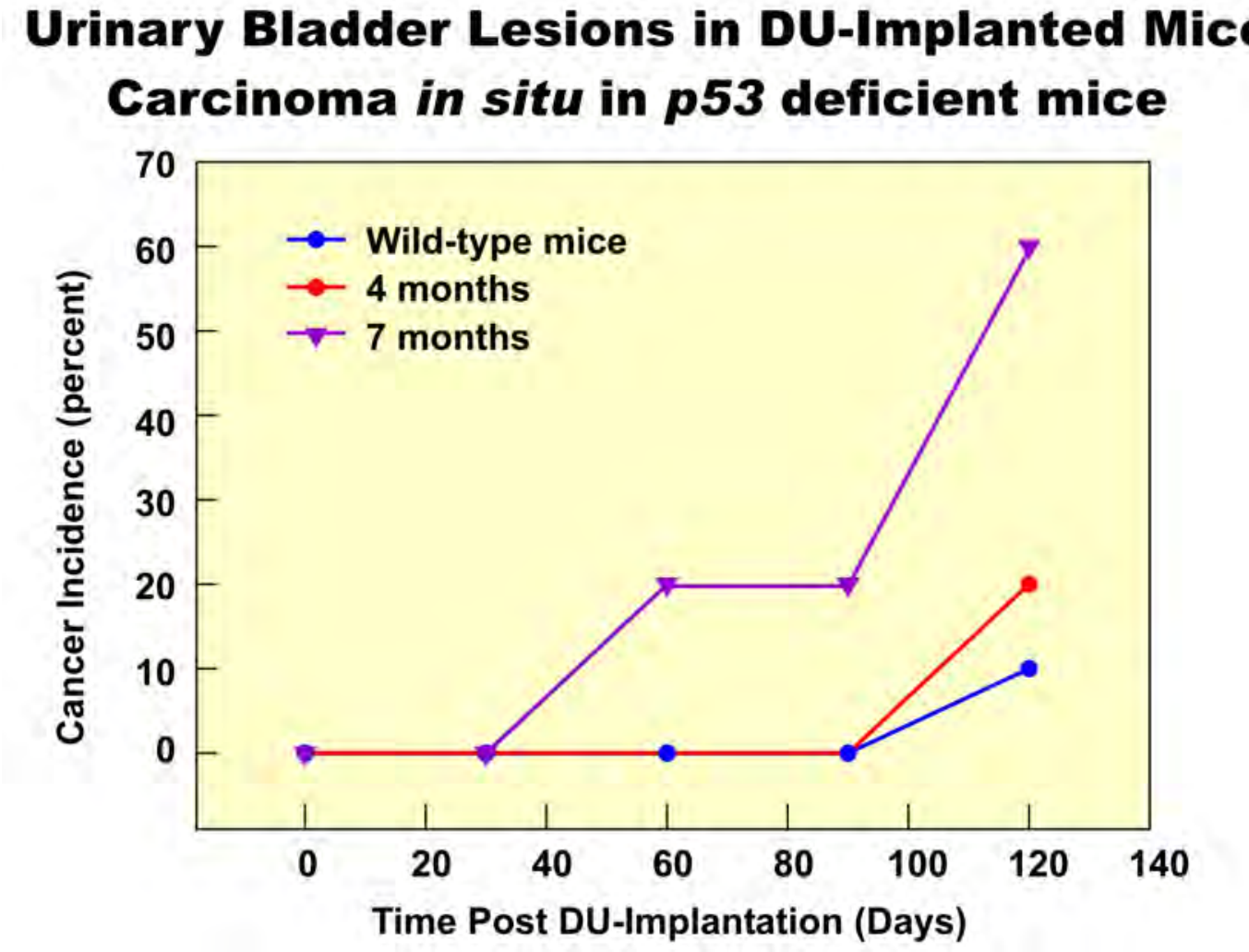
Mechanisms in Leukemia Induction
"Genetic versus Epigenetic"



Finding: DU Induces Leukemia in a Mouse Model

Miller et al., *Biochimie* 91(11) 112-115, 2009.

Another Model:
Bladder Carcinoma Model



Finding: Preliminary

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

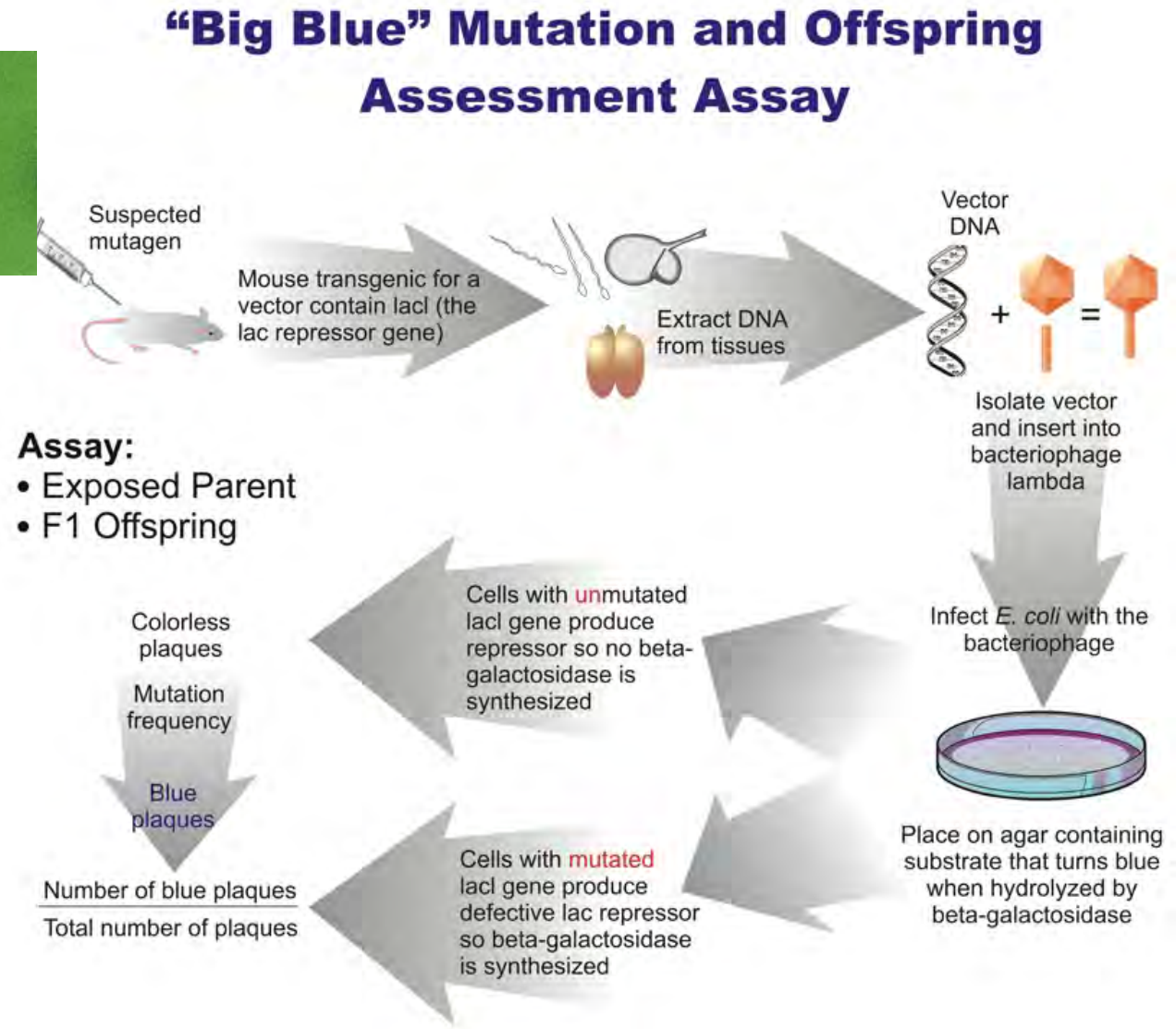
1) **Is long-term exposure to internalized DU carcinogenic?**

2) **Does DU cause transgenerational effects?**

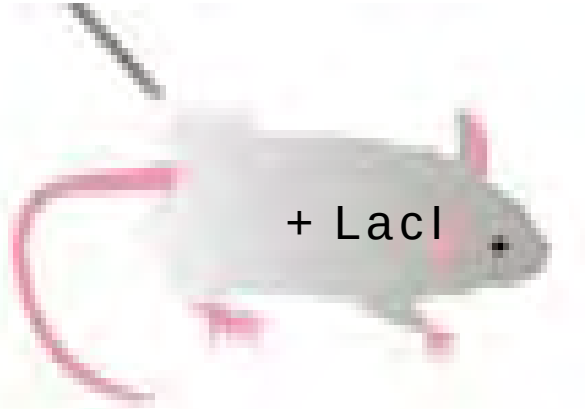
3) **Does DU cause radiation effects?**

4) **Can we distinguish between DU and other exposures (radiation, chemical)?**

Model to Assess Transgenerational Effects of Radiation or Heavy Metals



Implantation of DU Pellet



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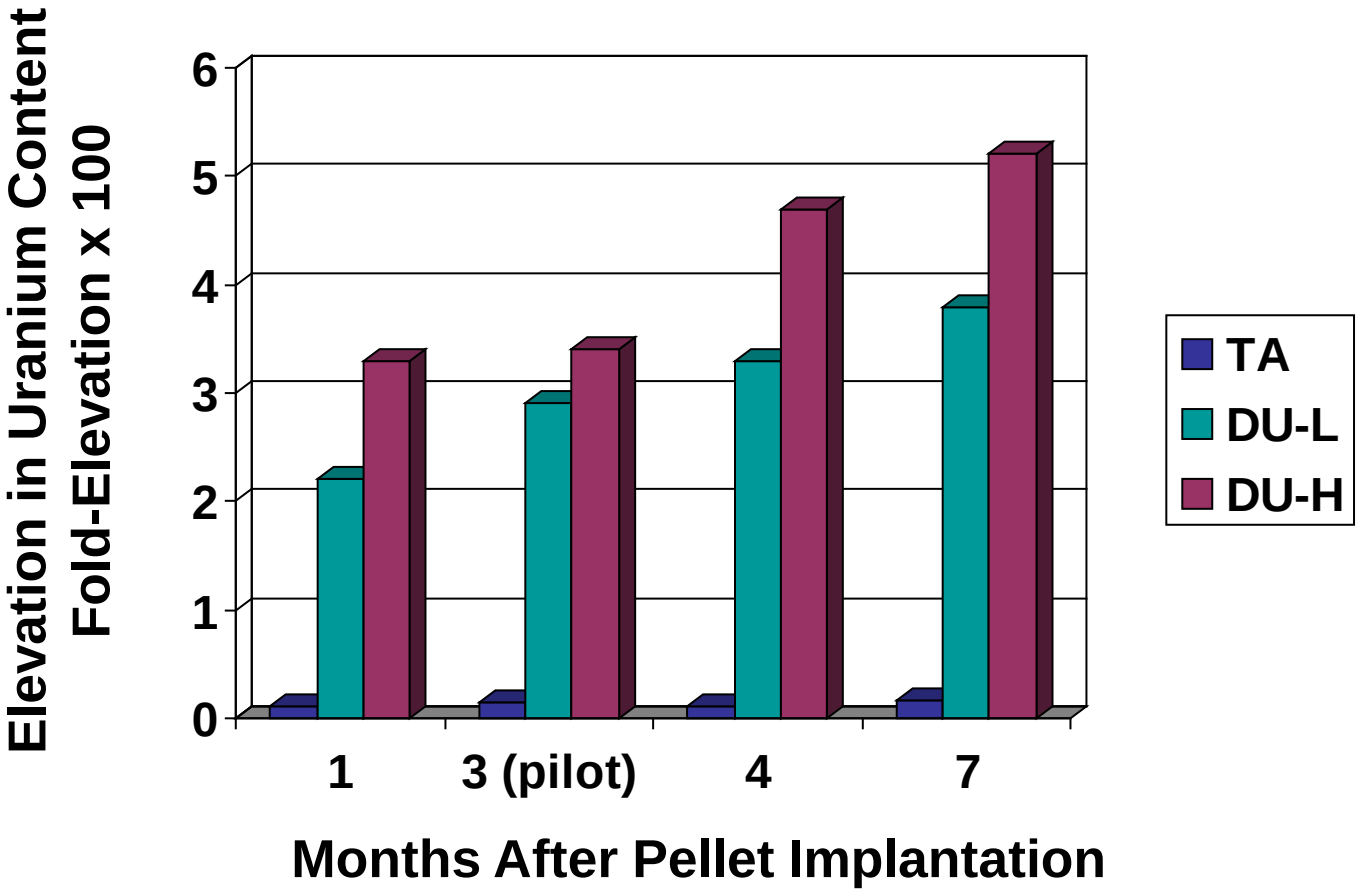
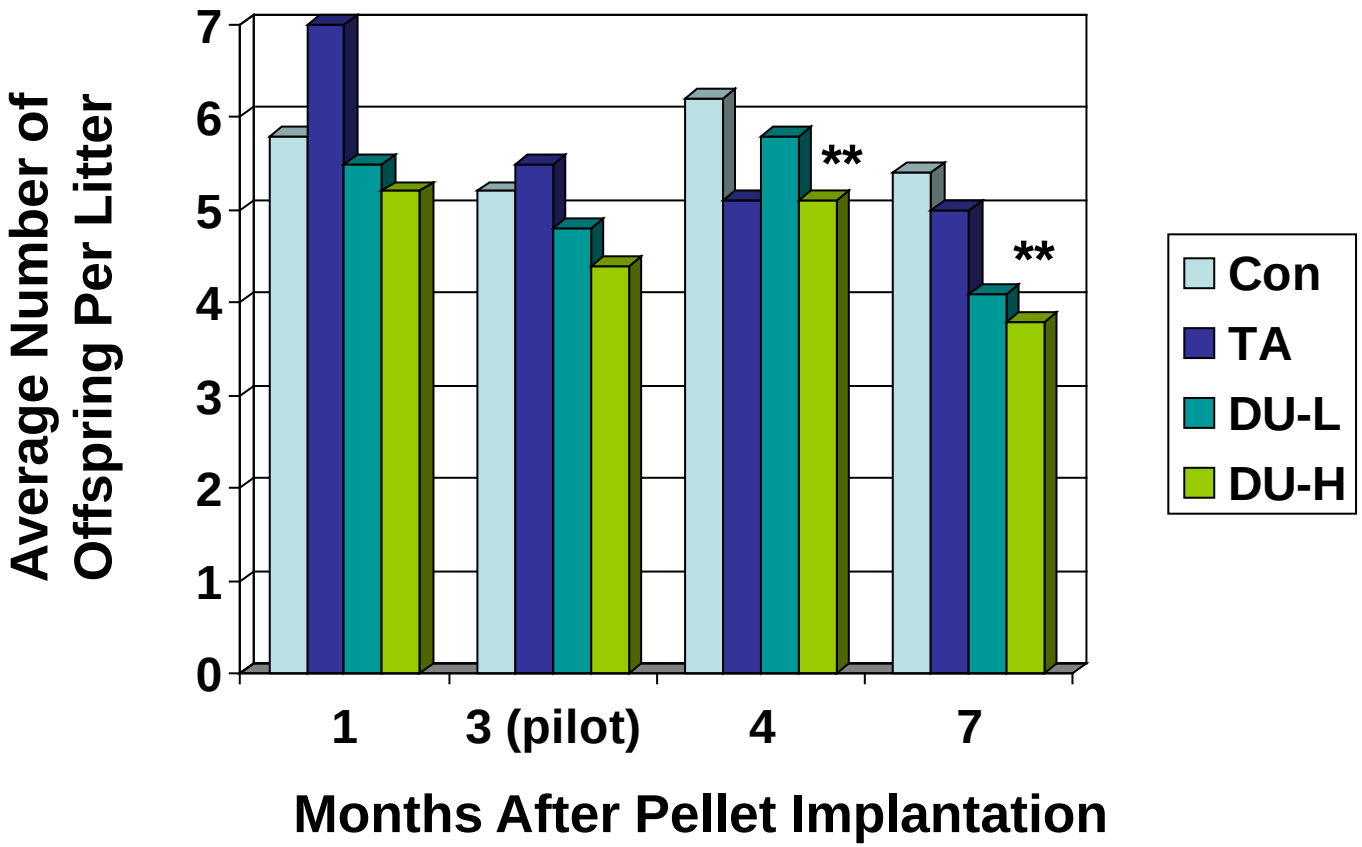
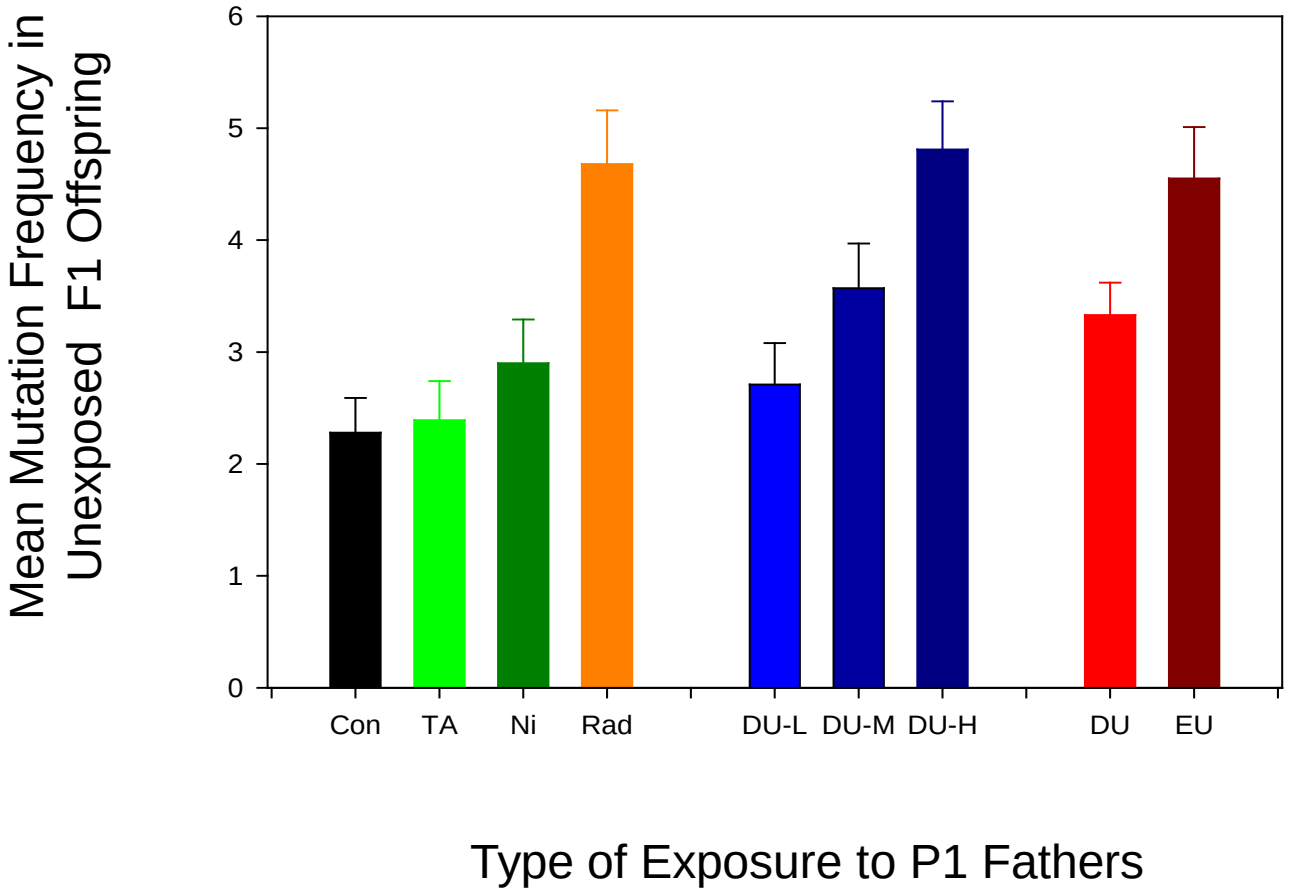
Effect of Paternal Exposure to DU on Testes Uranium Content



Effect of Paternal Preconceptional Exposure to DU on Litter Size



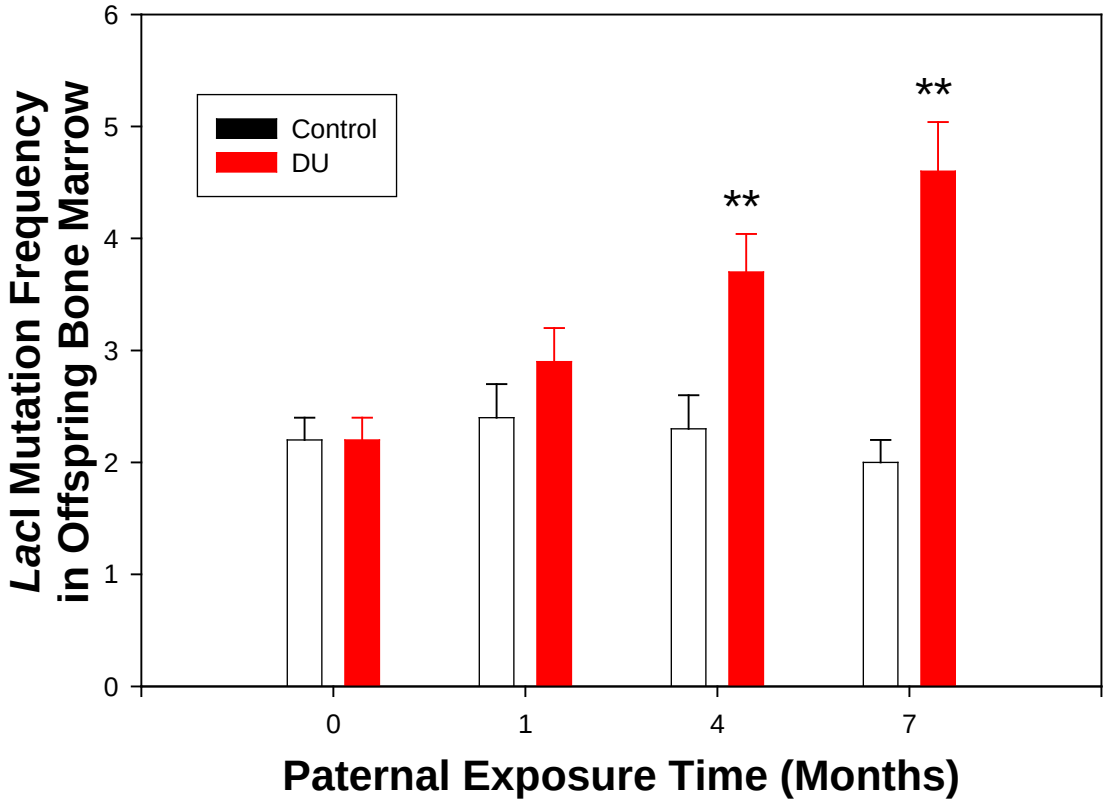
Transgenerational Effects of Depleted Uranium: Involvement of Radiation



Miller et al., 2010 *Health Physics*, epub Aug 30, 2010

Time-Dependence

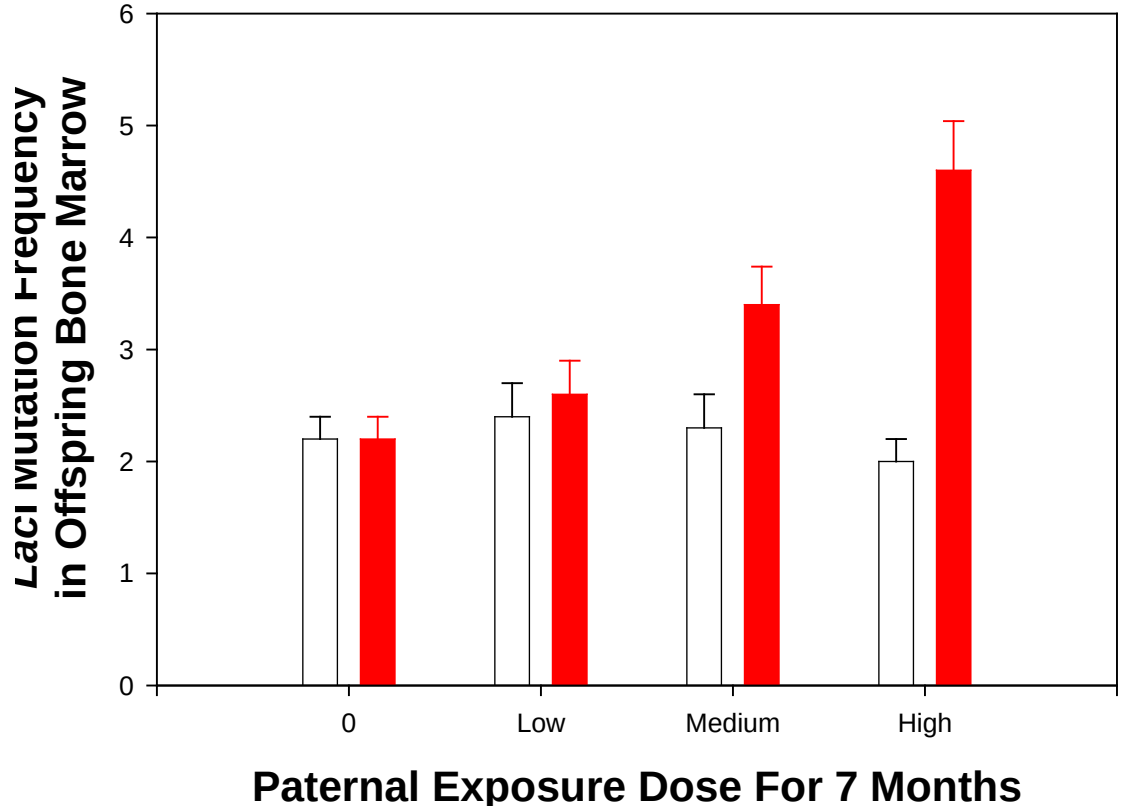
Mutation Frequencies in Bone Marrow of F1 Offspring Born to DU-implanted Male Mice (High dose)



***Studies were conducted with the support of the USAMRMC-CDMRP-PRMRP; DAMD17-02-0185*.**

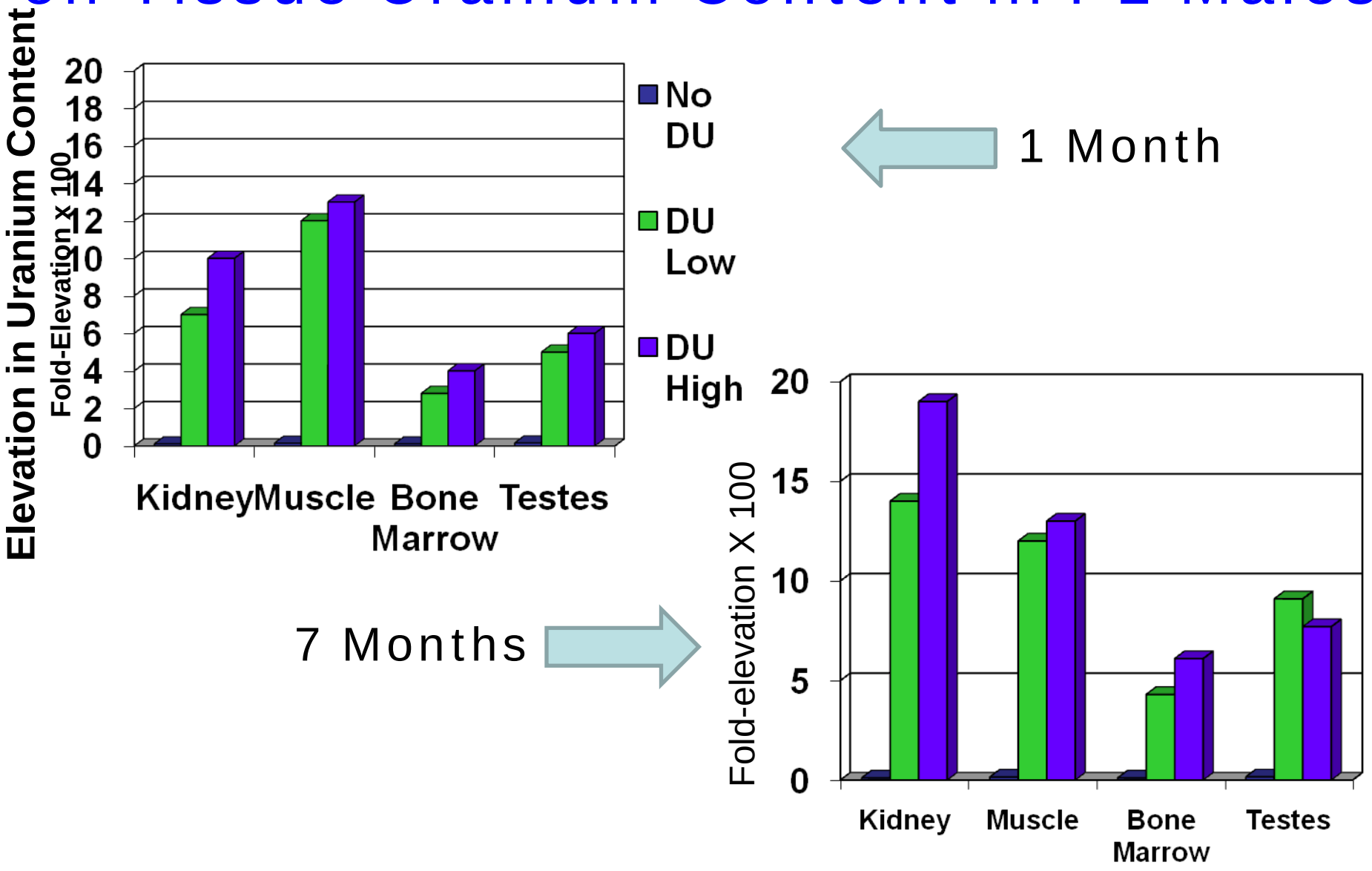
Dose-Dependence Transgenerational Effects *in Vivo*

Mutation Frequencies in Bone Marrow of F1 Offspring Born to DU-implanted Male Mice (7 Months)

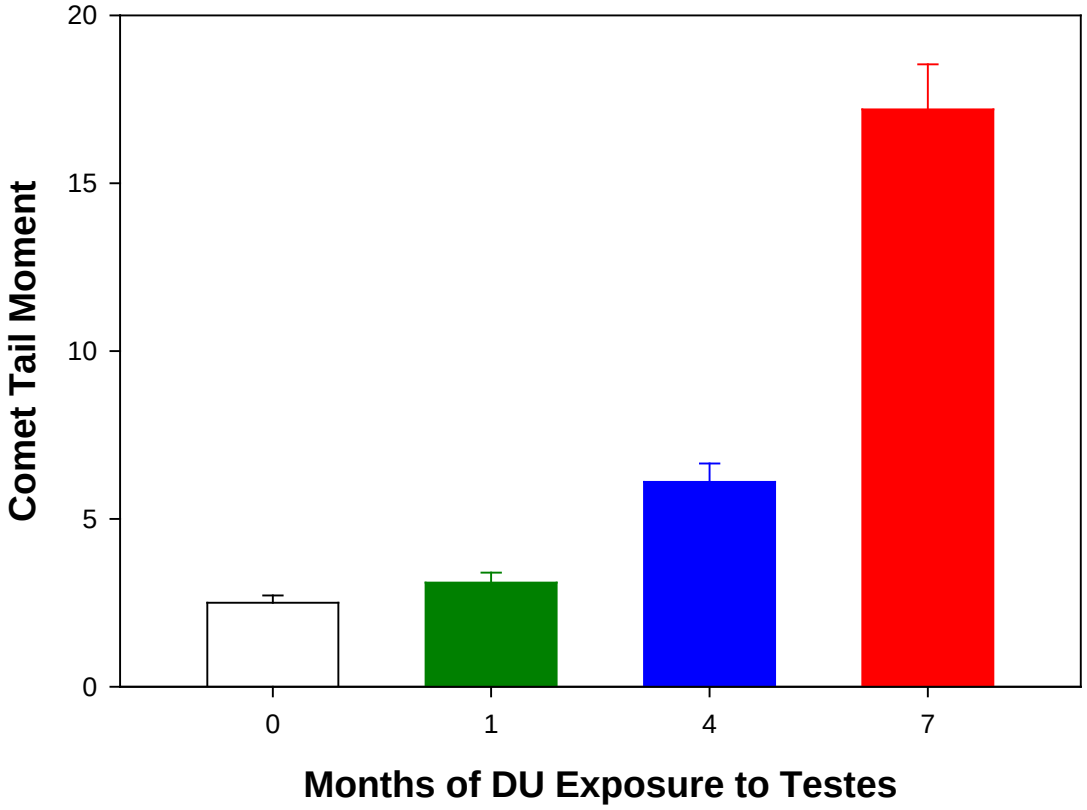


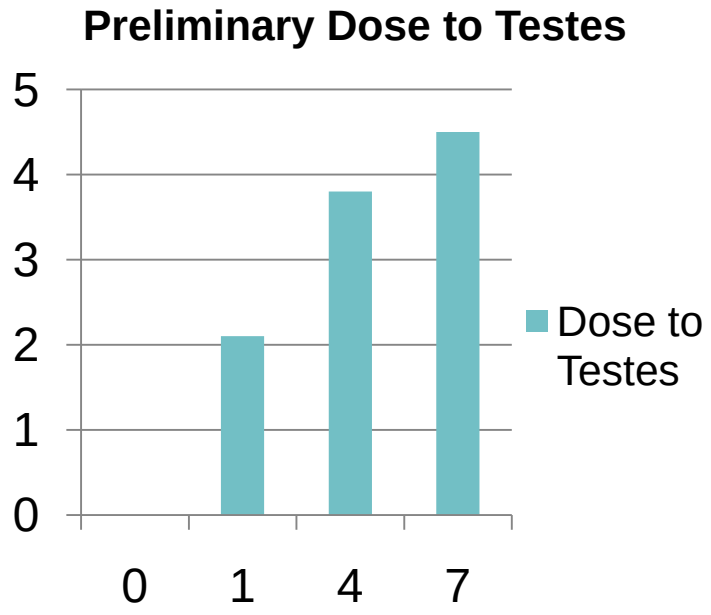
Studies were conducted with the support of the USAMRMC-CDMRP-PRMRP; DAMD17-02-0185.

Effect of Internalized Exposure to DU on Tissue Uranium Content in P1 Males

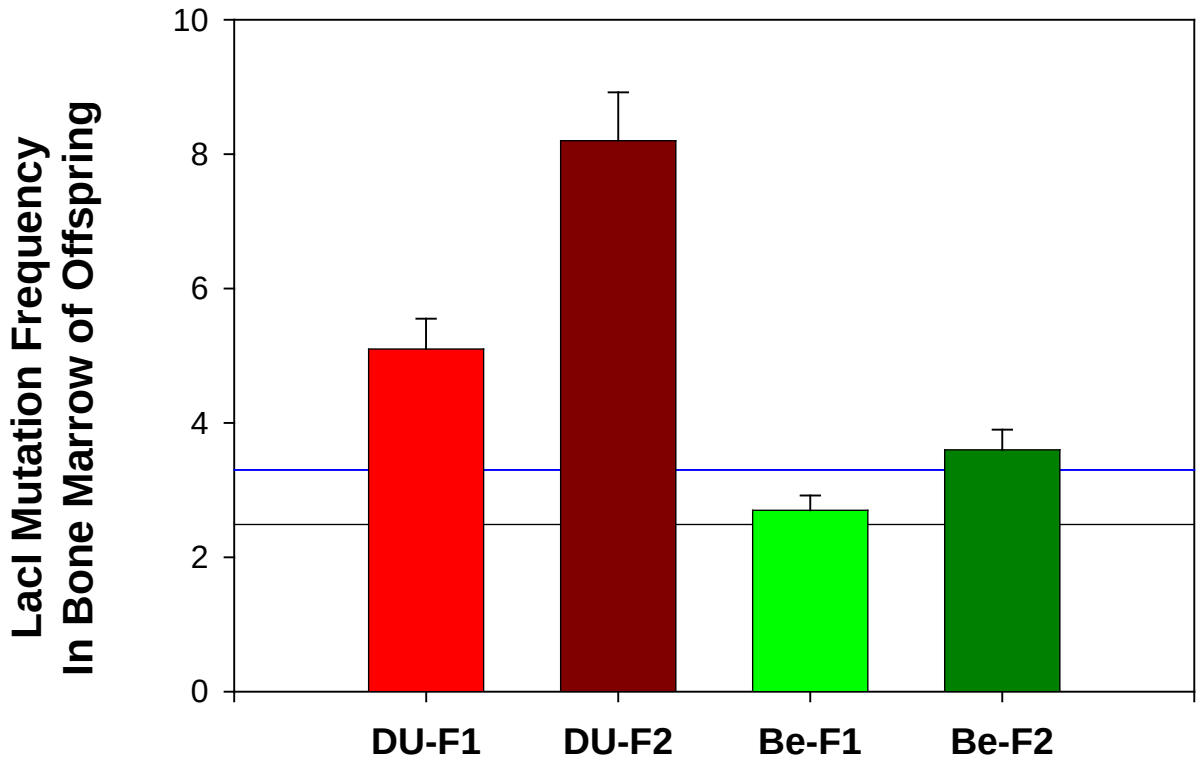


Germ Cell DNA Damage After Internalized DU Exposure in P1 Males





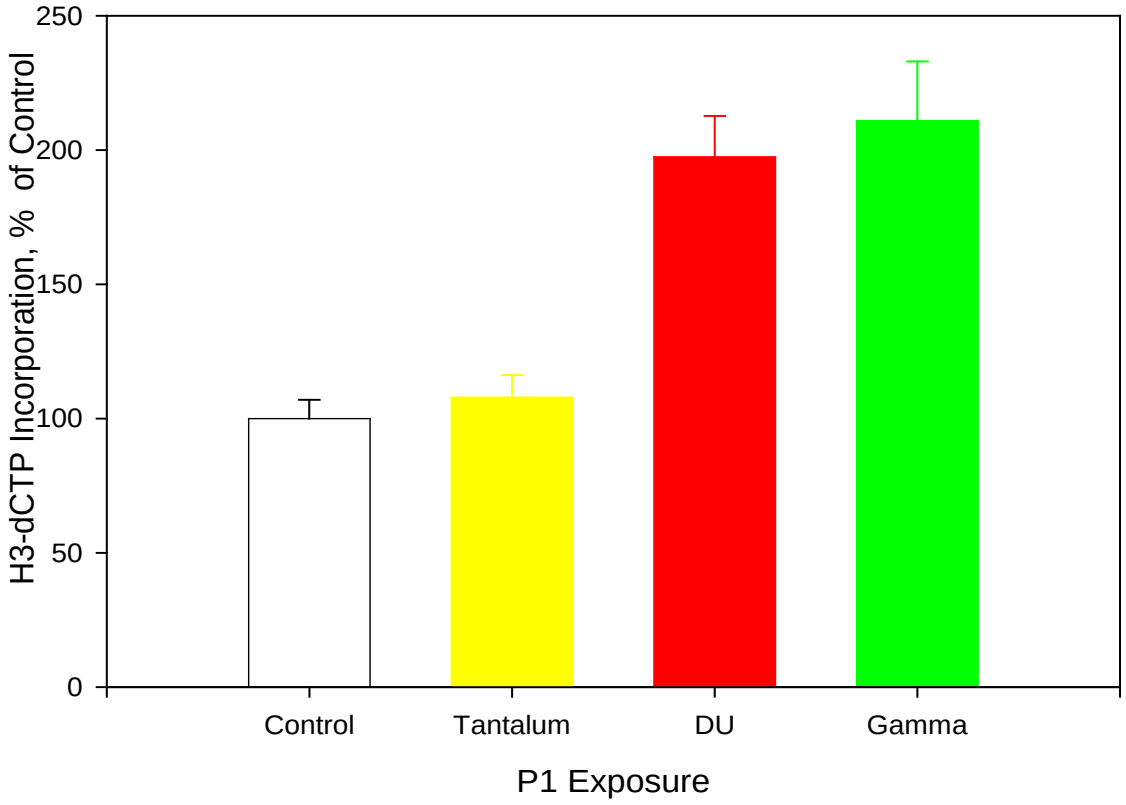
Mutation Frequencies in Bone Marrow of F2 Offspring Born to Heavy Metal-implanted Male Mice (High dose)



Lison, Monleau, Miller, in preparation

Epigenetic Mechanisms In Transgenerational DU Effects

Levels of DNA Methylation in Offspring Bone Marrow



Finding: Preconceptional Paternal DU Exposure Induces Genomic Instability in Unexposed Offspring

Finding: DU Internalized Exposure Induces Germ Cell DNA Damage

Evaluation of DU on Rat Behavior, Toxicological Endpoints, Male Reproduction

Uranium Distribution	Behavior Neurobio	Histopathology	Sperm Effects	Reproduction	Offspring Effects
Yes	No effects	No effects	No effects	No effects	F1 no cancers
Time and Dose Dependent					F1 ↑ Heart weight
					F1 unknown deaths assoc w P1 DU implants

Navy Medical detachment at Wright-Patterson Air Force Base

Arfsten DP, et al, 2005, Study of the reproductive effects in rats surgically implanted with depleted uranium for up to 90 days. *Toxicol Environ Health* A. Jun 11-25;68(11-12):967-97.

Arfsten DP, et al. 2006, Evaluation of the effect of implanted depleted uranium on male reproductive success, sperm concentration, and sperm velocity. *Environ Res.* Feb;100(2):205-15.

Arfsten DP, et al, 2007, Evaluation of the effect of implanted depleted uranium (DU) on adult rat behavior and toxicological endpoints. *J Toxicol Environ Health* A. 2007 Dec;70(23):1995-2010.

Arfsten DP, et al. 2009, Two-generation reproductive toxicity study of implanted depleted uranium (DU) in CD rats. *J Toxicol Environ Health* A.72(6):410-27.

Conclusions *in Vivo:*

1. **DU induces leukemia in a rodent model and is mutagenic in vivo.**

2. **DU induces transgenerational genetic instability in unexposed rodent/mouse offspring. Radiation plays a role in this process.**

3. **Reproductive toxicity has demonstrated conflicting results (oral versus embedded DU)**

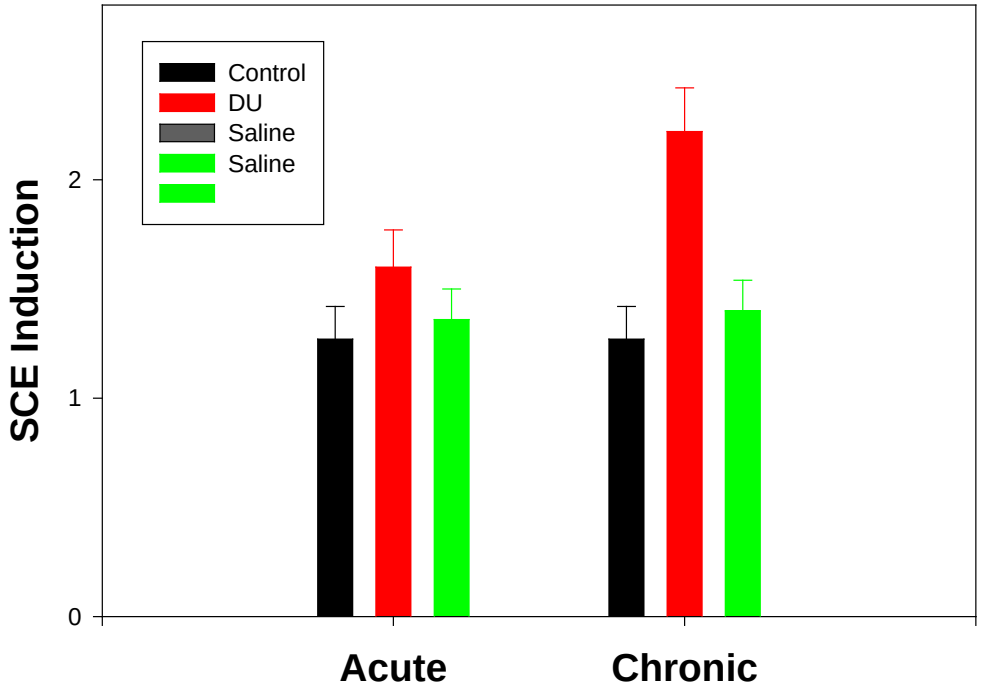
4. **DU Induces germ cell damage at high doses and long exposure times.**

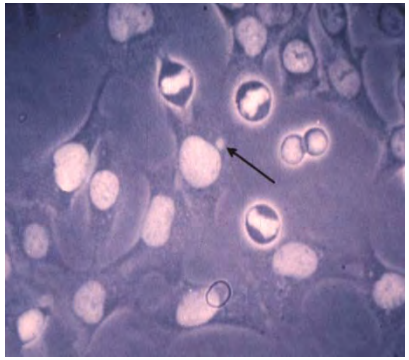
Additional Preliminary Studies

1. **Acute versus chronic**
2. **Inhalation versus chronic embedded exposure**
3. **Radiation effects in vivo**

Acute Versus Chronic Uranium Exposure

Comparison of Acute and Chronic DU Exposure: Induction of Micronuclei

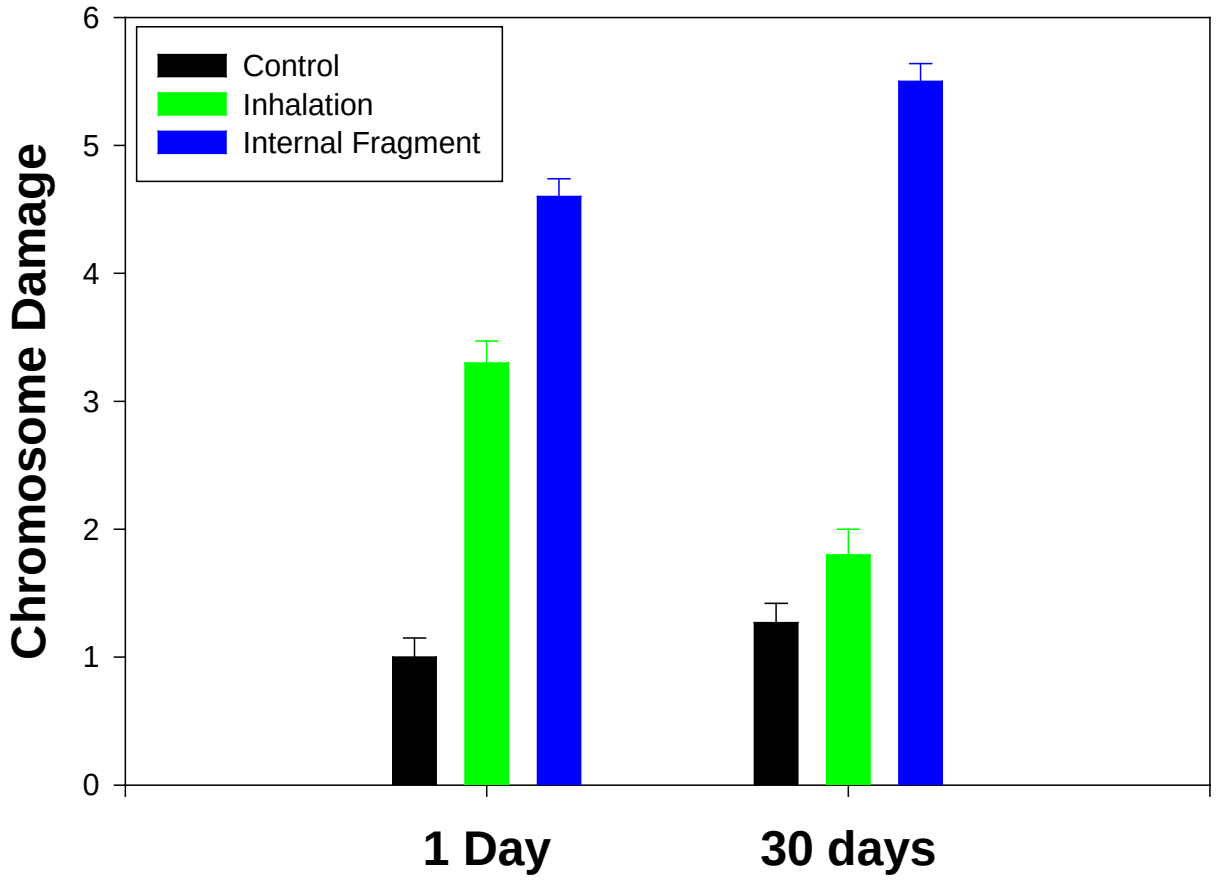




Acute = 350 mg/kg DU (1 injection); 14 days post injection
Chronic = 35 mg/kg DU (1x daily, 10 days); 4 days post final injection

Unpublished Data

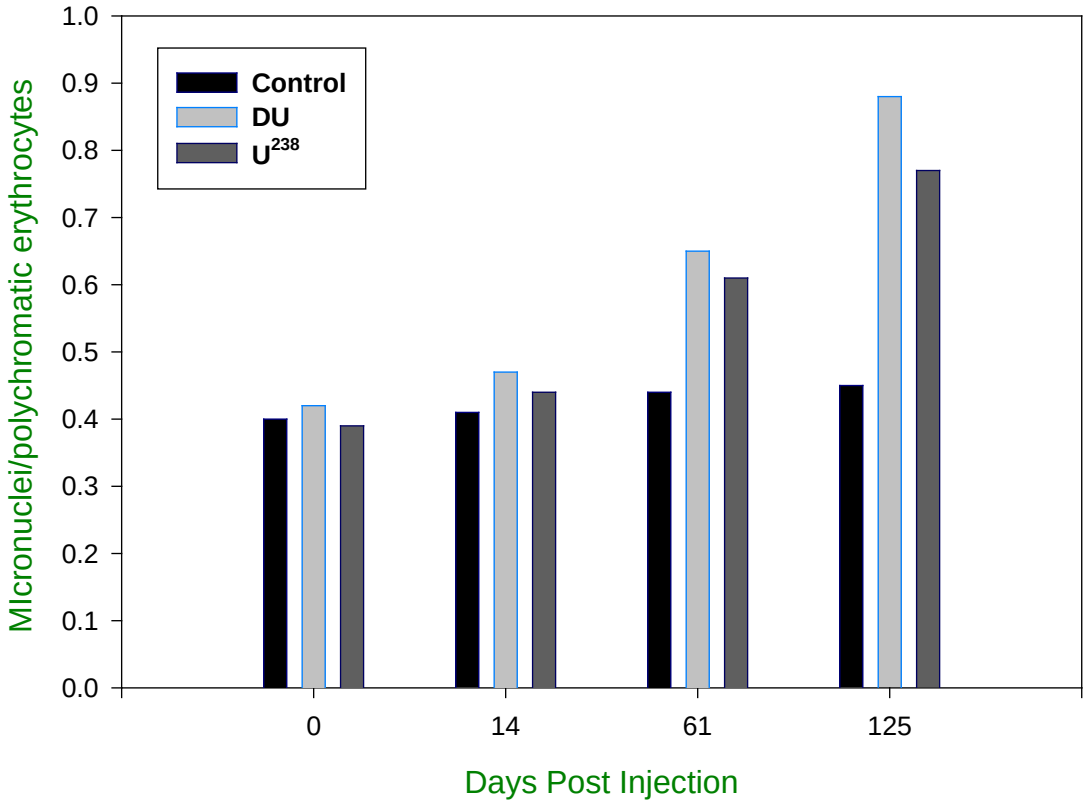
Comparison of Inhalation versus Chronic Internal Fragment Exposure *in vivo*: Measurement of Chromosomal Damage



Unpublished data

Radiation Effects *In Vivo*

Comparison of DU- and ^{238}U- Uranyl Nitrate *in vivo*: Induction of Micronuclei



Unpublished data

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

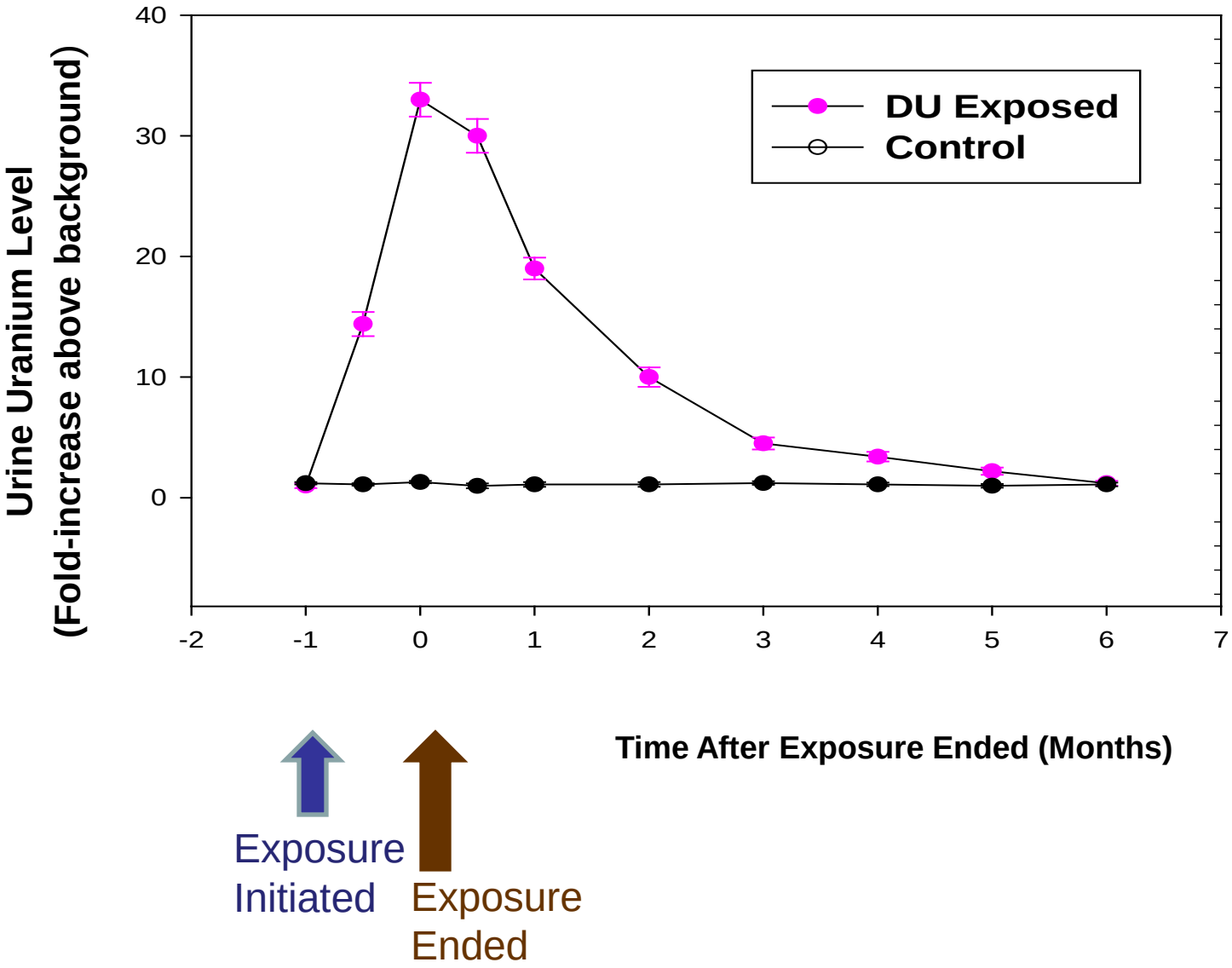
1) **Is long-term exposure to internalized DU carcinogenic?**

2) **Does DU cause transgenerational effects?**

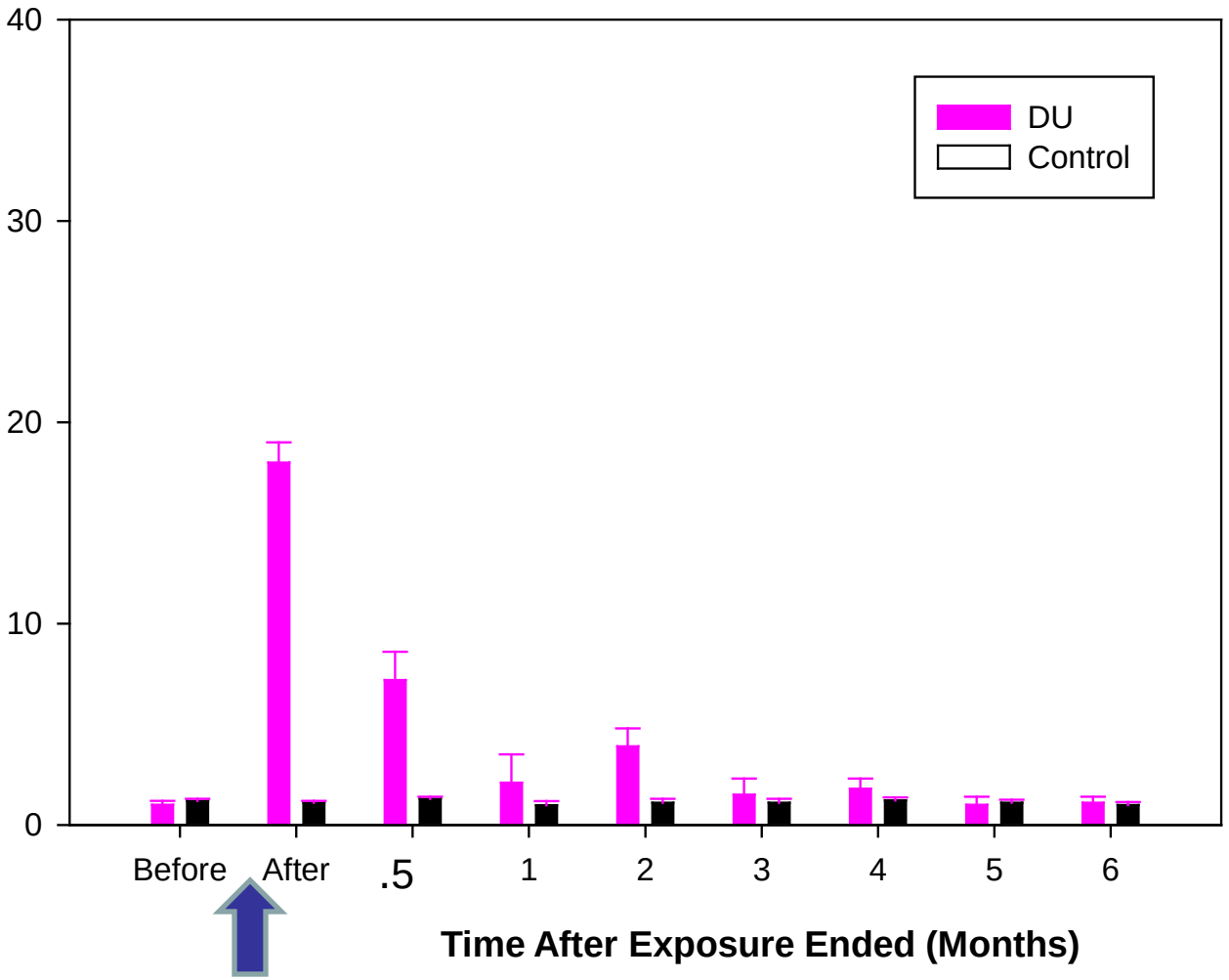
3) **Does DU cause radiation effects?**

4) **Can we distinguish between DU and other exposures (radiation, chemical)?**

Timecourse of Urine Uranium Content Following Internalized/Embedded DU Exposure



Timecourse of Urine Uranium Content Following DU Inhalation



Monleau, *Inhalation Toxicol*. Oct 18(11), 2006.

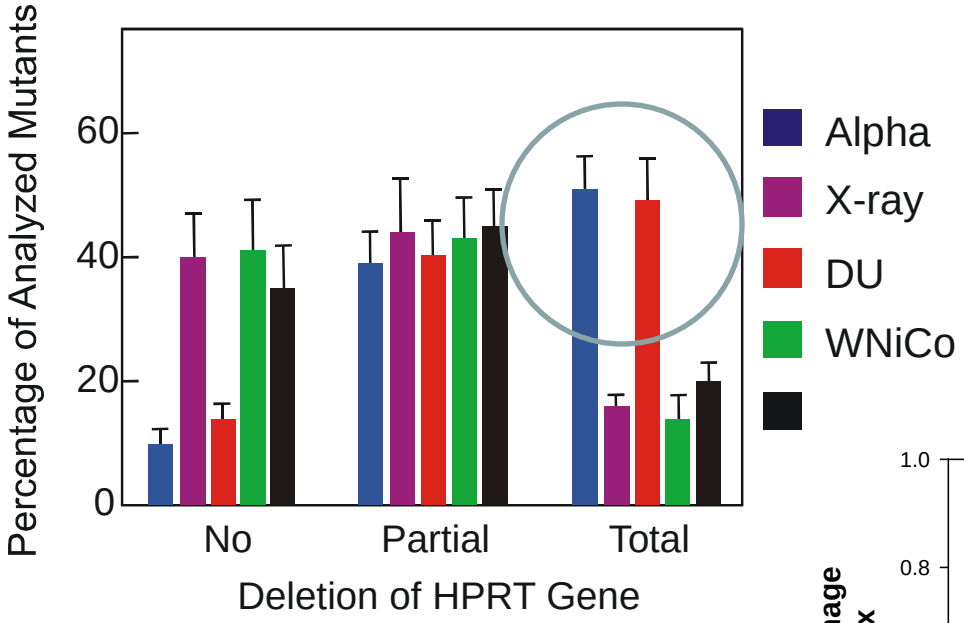
Is there a better exposure marker?

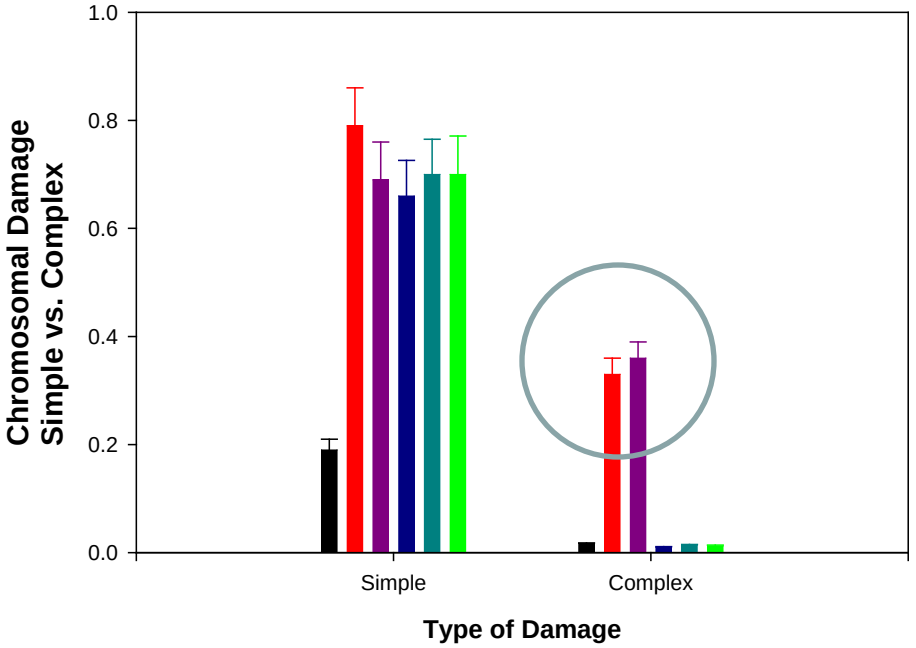
Good Marker Characteristics

1) **Marker specificity**

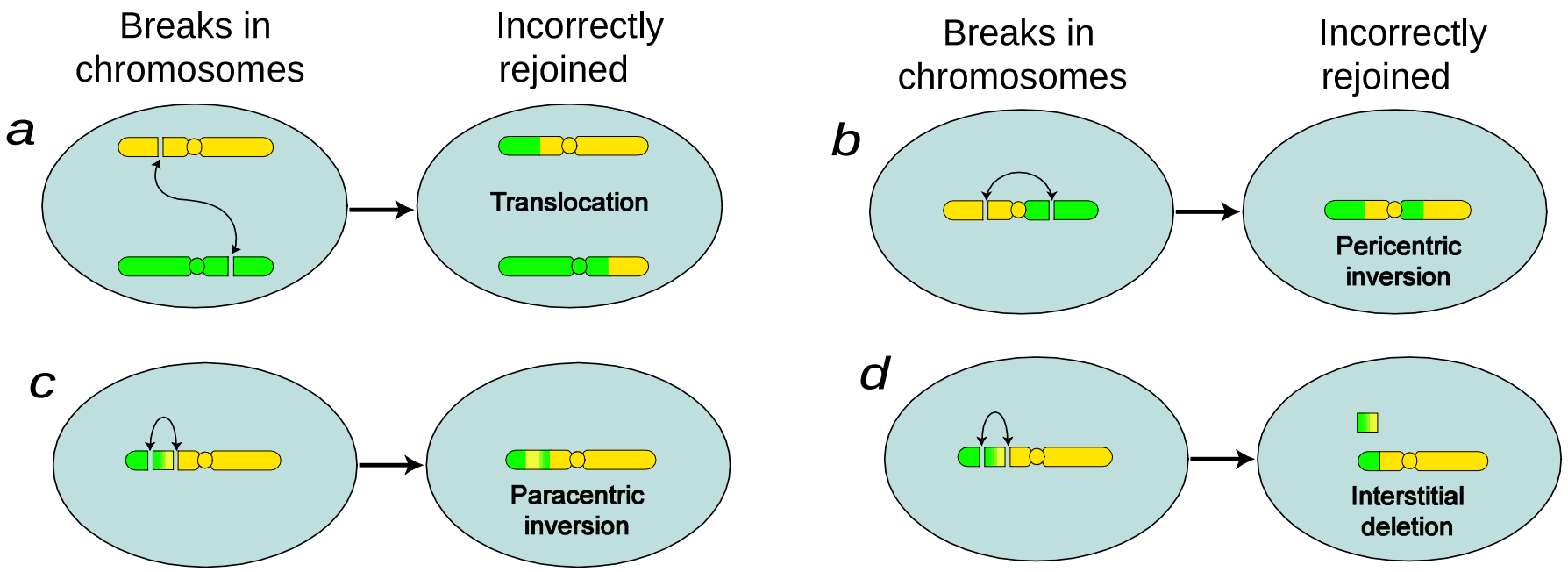
2) **Marker persistence**

DU and Alpha Particles Cause Similar Damage



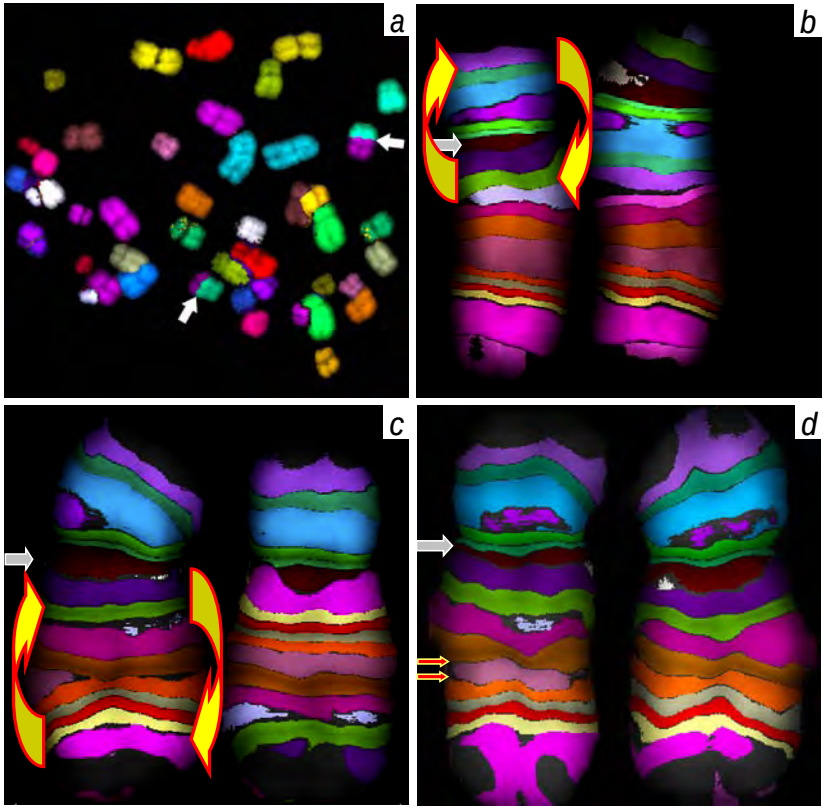


Chromosomal Damage as an Alpha Particle Exposure Biomarker



1. **(*a*)** inter-chromosomal aberration produced by misrejoining of chromosome breaks on two different chromosomes
2. **(*b-d*)** intra-chromosomal aberrations produced by misrejoining of breaks **(*b*)** on two different arms of a single chromosome, or **(*c-d*)** within a single chromosome arm. *Intra*-chromosomal aberrations generally originate from pairs of chromosome breaks that are closer together than those producing inter-chromosomal aberrations:

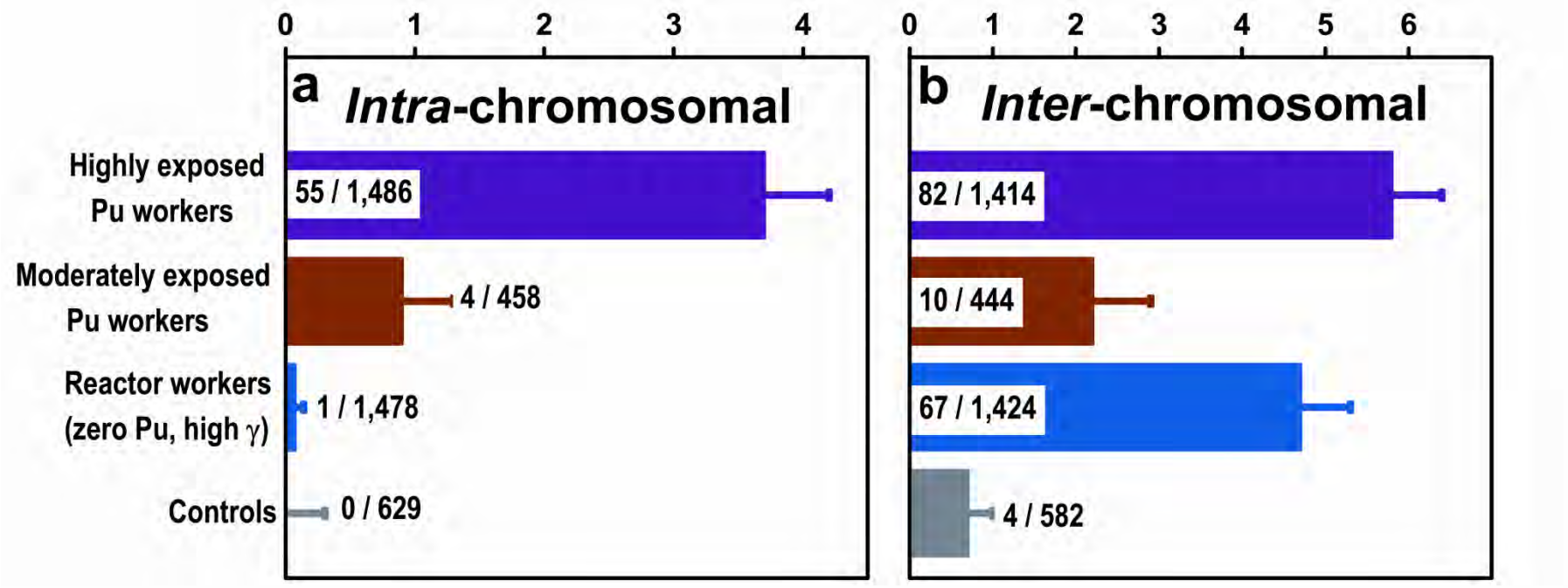
Development of Assay to Measure Stable Inter- and Intra- Chromosomal Aberrations in Workers Exposed to Alpha Particles Mayak Plutonium Workers



a) Inter-chromosomal aberration (simple translocation),
b) Inter-arm aberration (pericentric inversion)
c) Intra-chromosomal intra-arm aberration (paracentric inversion)
d) Intra-chromosomal intra-arm aberration (intra-arm deletion)

Brenner, Hande 2003, *Am. J. Hum. Genet.*, 72:1162-1170.

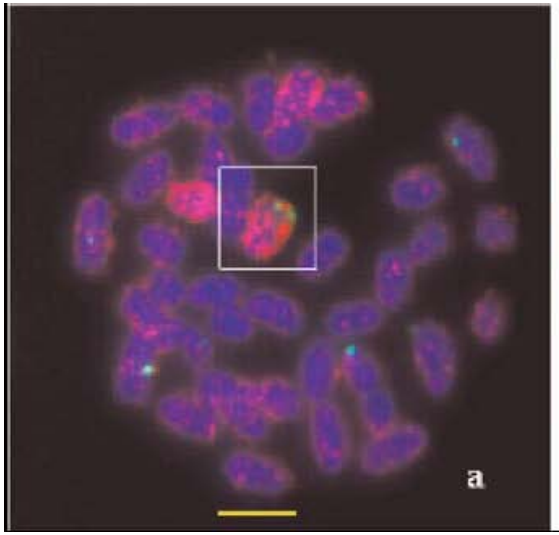
Measured Yields of Stable Chromosomal Aberrations In Peripheral Blood Lymphocytes Of Mayak Workers



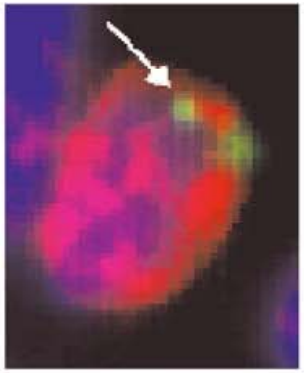
Need a large number of samples

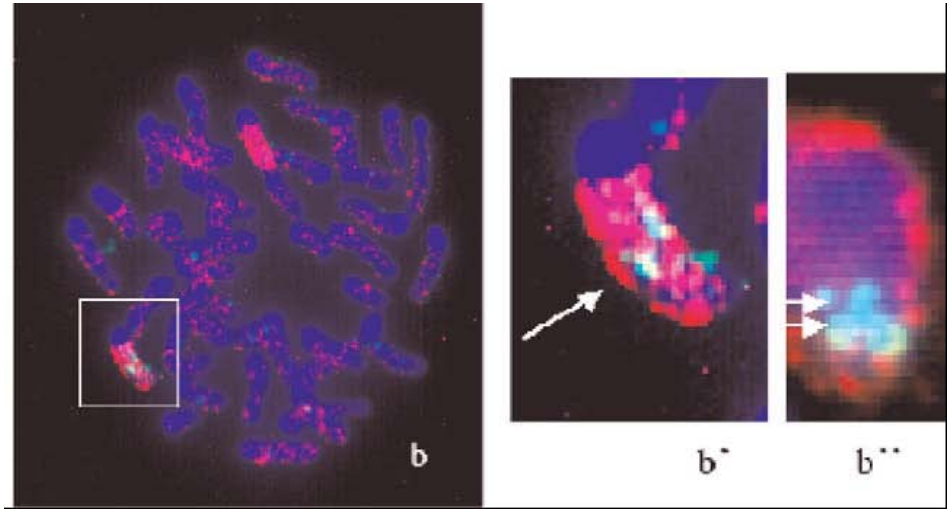
Brenner, Hande 2003, *Am. J. Hum. Genet.*, 72:1162-1170.

Instability of Mouse Chromosome 11: Rearrangement of the telomeric region in Mouse Samples



Sub-telomeric probe

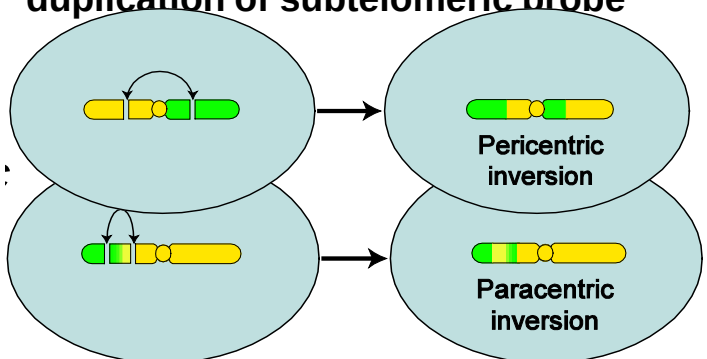




Exposure	Insert Chr 11	Translo Chr 11	Invers Chr 11
DU	16/100	10/100	10/100
^{60}Co Gamma	5/100	5/100	0
Tungsten alloy	6/100	6/100	0
Ethyl benzene	4/100	8/100	0

FISH with a subtelomeric probe for Mouse chromosome 11.

a Metaphase normal chr 11
a' enlargement of Chr 11; 2 subtelomeric signals
b elongated chr; inverted duplication
b' green probe seen as doublet
b'' duplication of subtelomeric probe



Conclusions *in Vivo:*

Internalized chronic DU exposure in vivo:

1. **- causes uranium re-distribution to multiple organs.**
2. **- Is associated with urine mutagenicity**
3. **- Induces chromosomal damage**
4. **- Indices leukemia development in mice**
5. **- causes preconceptional paternal exposure to induce genomic damage in unexposed offspring**
6. **- Induces germ cell DNA damage**

These results indicate that the responses are DU dose- and time- dependent

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SGT Tyson, USA

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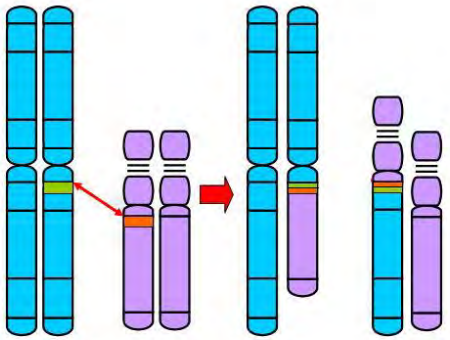
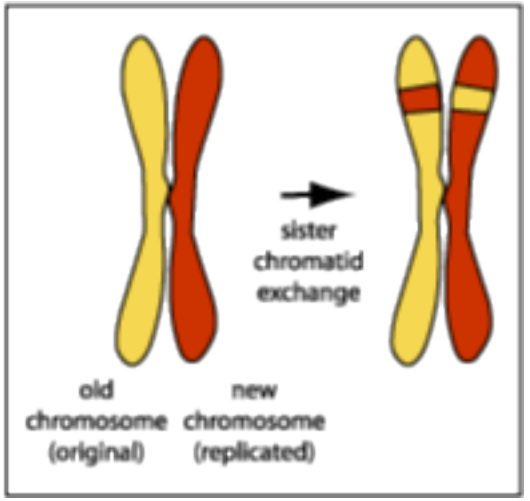
Other Biomarkers

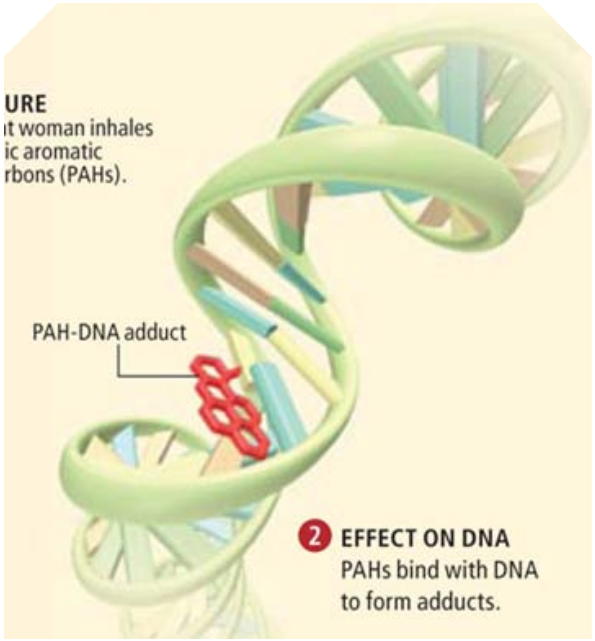


Translocations

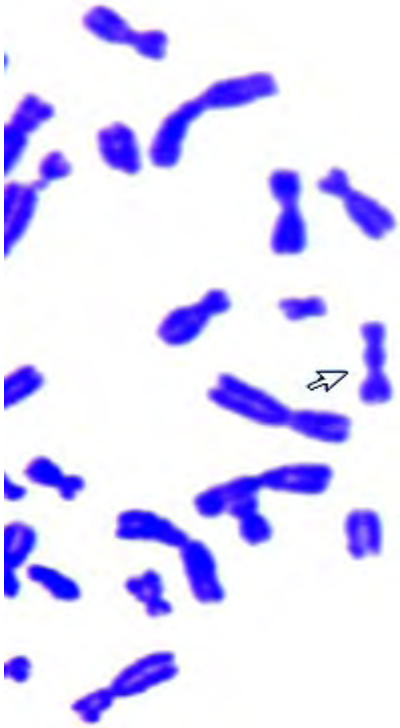
Sister Chromatid Exchange



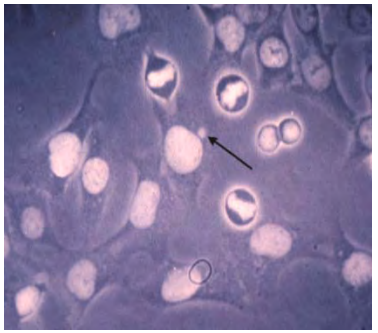
DNA Adducts



Dicentrics



Micronuclei

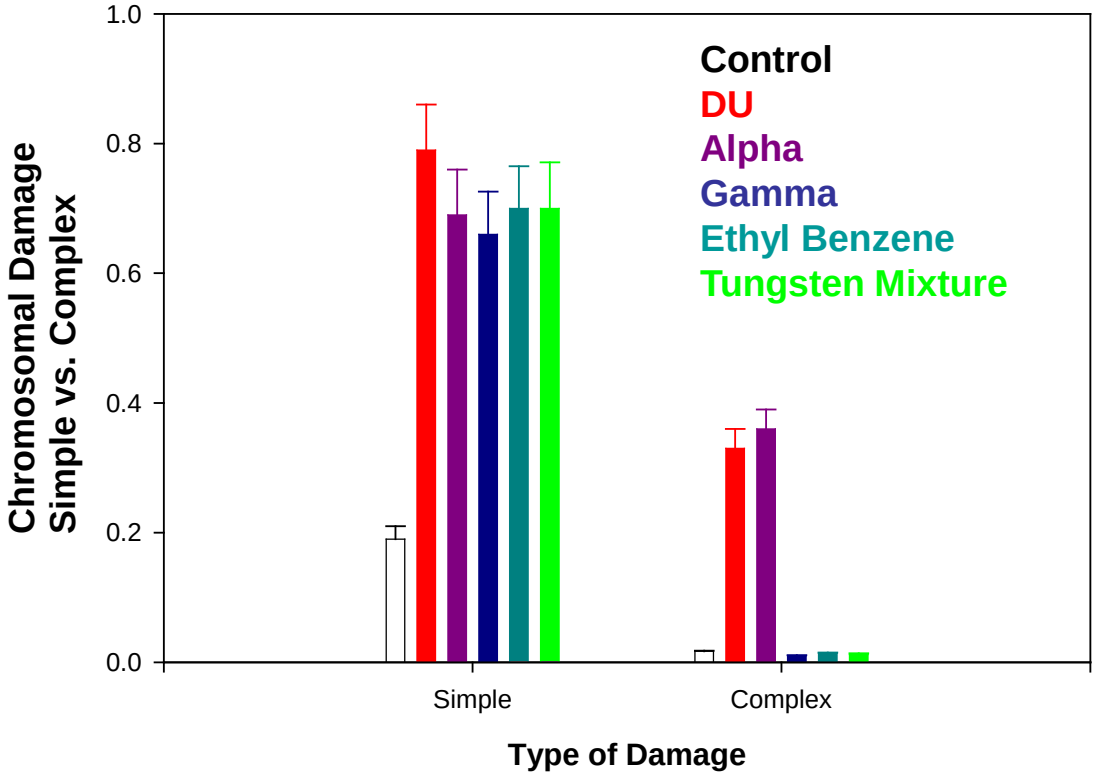


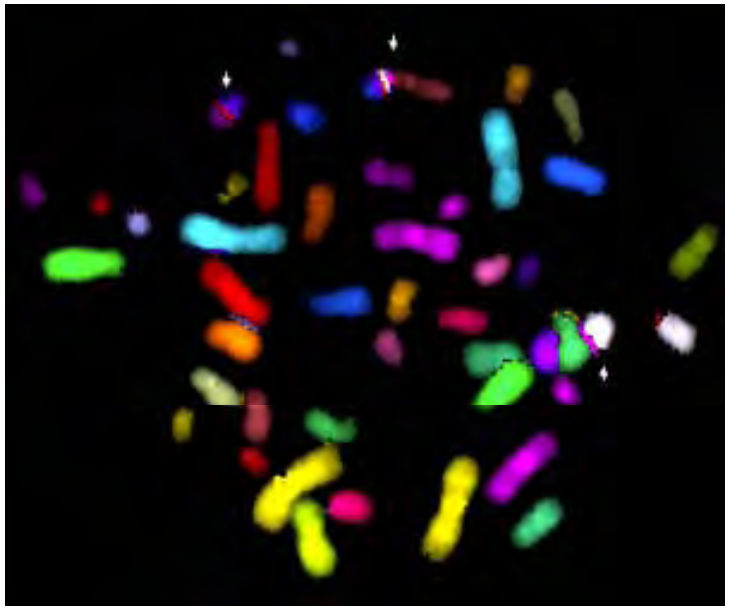
Simple vs Complex Damage

Simple: 1 or 2 breaks in 1 chromosome

Complex: 3 or more breaks in 2 or more chromosomes

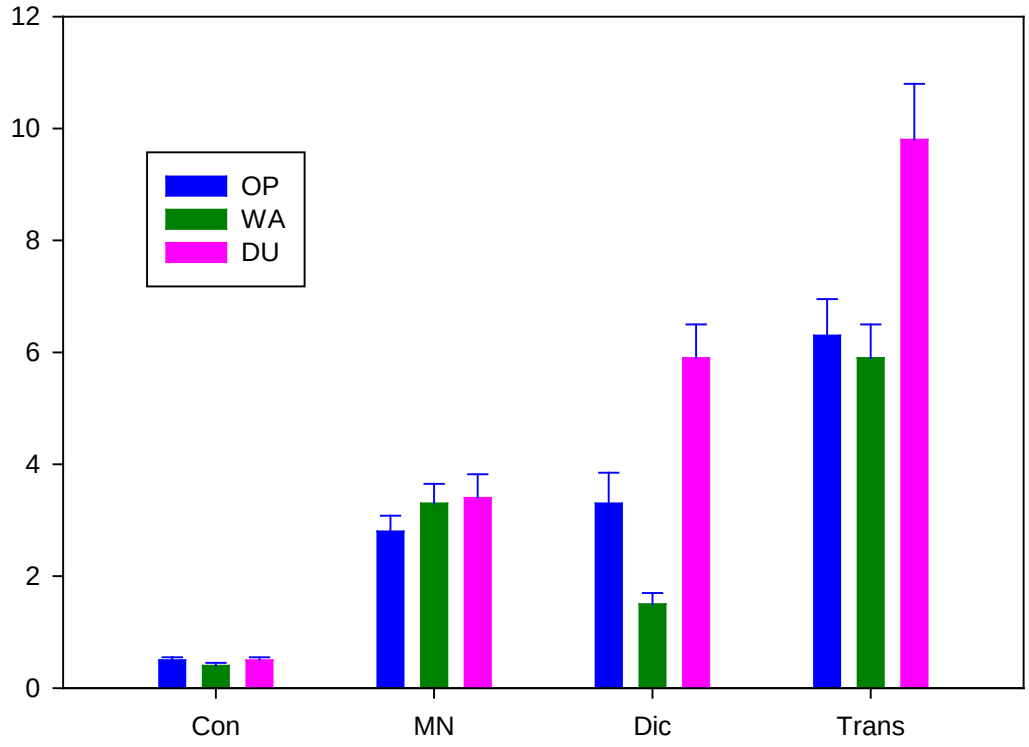
Chromosomal Damage in Human Osteoblast Cells Exposed to DU, Alpha Particles, Gamma Radiation, Tungsten Mixture, Ethyl Benzene *in vitro*

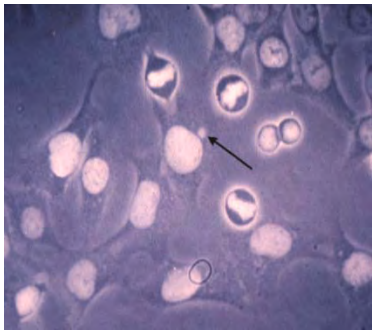


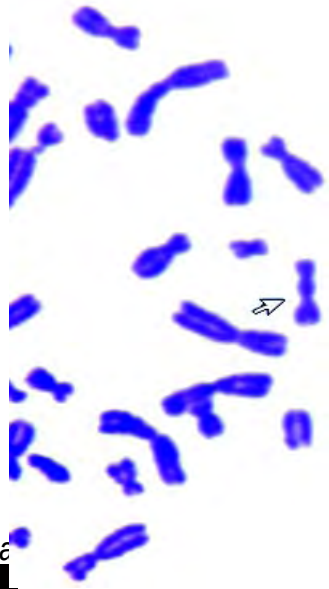


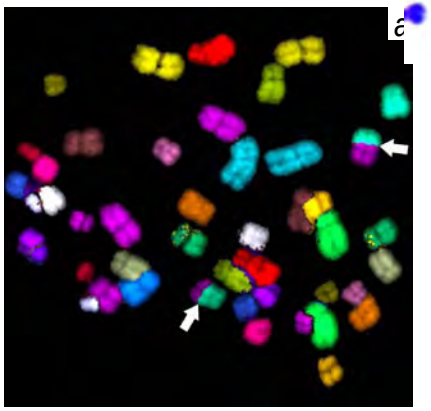
Rona Anderson, MRC
and Patrick Lavelle, Univ of Paris

Early Cytogenetic Damage in HOS Cells: Organophosphate, Tungsten Alloy, Depleted Uranium



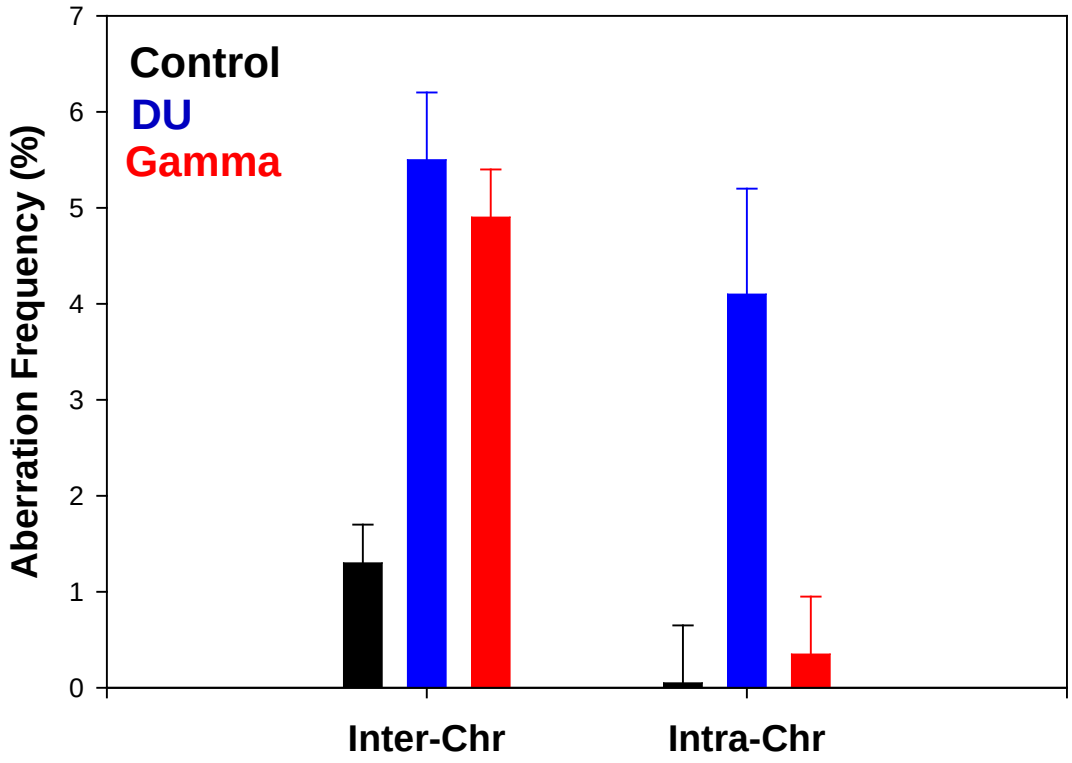






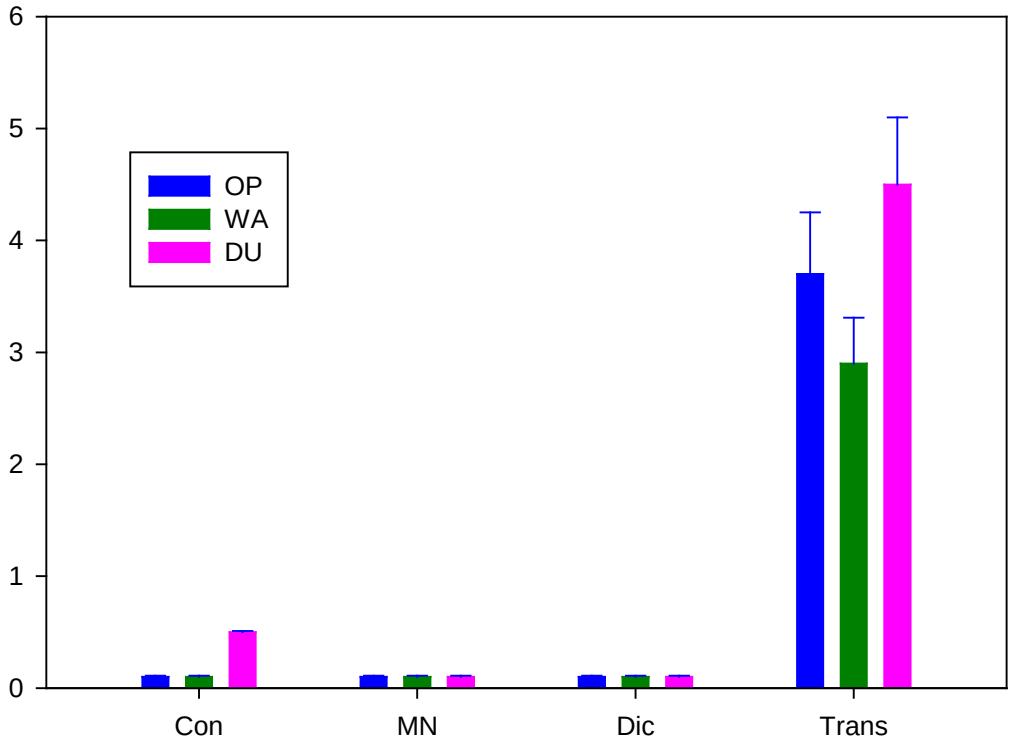
mBAND Assay

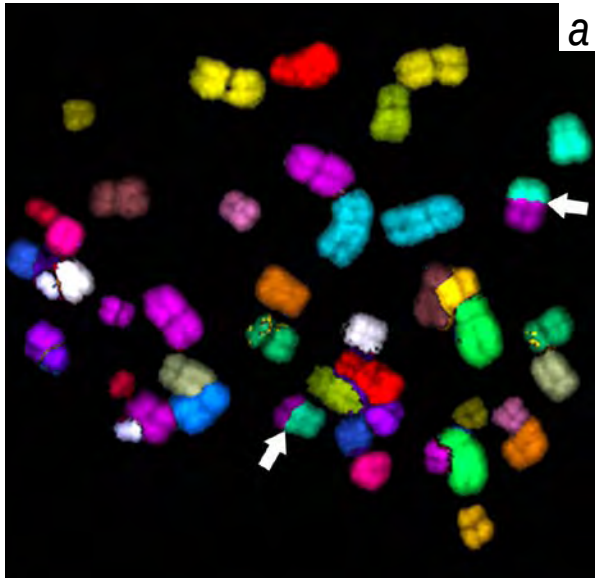
Measured Yields of Stable Chromosomal Aberrations *in vivo*



Persistence and Specificity

Persistent Cytogenetic Damage in HOS Cells: Organophosphate, Tungsten Alloy, Depleted Uranium





Chromosomal Translocations are similar